Synthesis and Biological Evaluation of Neutral and Zwitterionic 3-Carboranyl Thymidine Analogues for Boron Neutron Capture Therapy

Youngjoo Byun,[†] Junhua Yan,[†] Ashraf S. Al-Madhoun,[‡,#] Jayaseharan Johnsamuel,[†] Weilian Yang,[§] Rolf F. Barth,[§] Staffan Eriksson,[‡] and Werner Tjarks*,[†]

Division of Medicinal Chemistry & Pharmacognosy in College of Pharmacy and Department of Pathology, The Ohio State University, Columbus, Ohio 43210, Department of Molecular Biosciences, Swedish University of Agricultural Sciences, The Biomedical Center, Uppsala, SE-75123 Uppsala, Sweden

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Novel 3-carboranyl thymidine analogues (3CTAs) were synthesized as potential boron delivery agents for boron neutron capture therapy (BNCT). This library includes six zwitterionic NH_3^+-*nido-m*-carborane-substituted thymidine analogues (Thds) and the corresponding neutral NH_2-*closo-m*-carborane-substituted counterparts. All compounds of this library were good substrates for recombinant human thymidine kinase 1 (TK1) with phosphorylation rates up to 89% relative to that of Thd. One compound out of this library, 3-[3-(7-NH_3^+-*nido-m*-carboran-1-yl)propan-1-yl]thymidine (**19b**), showed selective retention in TK1-expressing murine L929 wild-type tumors versus L929 TK1 (−) tumors in biodistribution studies. The biological evaluation of the zwitterionic NH_3^+-*nido-m*-carborane-substituted Thds indicated improved aqueous solubility and similar or even superior potential as BNCT agents compared with different classes of 3CTAs (*Cancer Res*. **2004**, *64*, 6280−6286 and 6287−6295). To complete previous structure−activity relationship (SAR) studies, 3-[(*closo-o*-carboranyl)methyl]thymidine (**4**) was also synthesized and evaluated.

Introduction

Boron neutron capture therapy (BNCT) is a binary system for the treatment of cancer that is based on the nuclear reaction of thermal neutrons with ^{10}B producing high linear energy transfer (LET) $^4He^{2+}$ (α) and $^7Li^{3+}$ particles. These particles have a range of only 5−9 μm, which is approximately the size of one cell diameter. Provided ^{10}B-containing compounds were taken up selectively by the tumor cells, irradiation with thermal neutrons would kill the tumor cells without producing damage to healthy adjacent cells. For BNCT to be effective, high concentrations of boron in the tumor (15−30 μg/g) and ideally tumor to normal tissue ratios of >5−10:1 should be achieved. Thus, the development of efficient tumor-selective ^{10}B delivery system is of pivotal importance for successful BNCT.[1]

Boronated nucleosides, in particular derivatives of thymidine (Thd) and 2′-deoxyuridine (dUrd), have been intensively studied as BNCT agents because of their potential metabolic incorporation into tumors cells.[2−18] The key regulatory enzyme in the metabolism of boronated Thd/dUrd analogues presumably is cytosolic thymidine kinase 1 (TK1), which catalyzes the 5′-monophosphorylation of endogenous Thd and dUrd. The expression of TK1 is tightly cell cycle-regulated and active enzyme is found only in S-phase.[19] Therefore, conversion of boronated Thds/dUrds to the corresponding nucleotides by TK1 may result in their selective intracellular entrapment in tumor cells due to the attained negative charge.

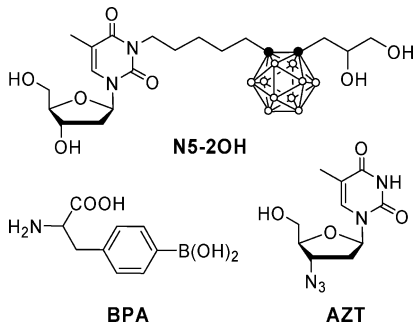

Figure 1. Structures of **N5-2OH**, BPA, and AZT.

Research activities in our laboratories have focused on the synthesis and biological evaluation of a specific class of boronated Thds, namely 3-carboranyl thymidine analogues (3CTAs), which proved to be excellent substrates of TK1.[2−6] In particular one 3CTA, designated **N5-2OH** (Figure 1), exhibited high uptake and retention in TK1-expressing wild-type tumor cells in vitro and preferential uptake in TK1-expressing wild-type tumors in vivo compared with the respective TK1 negative counterparts.[20,21] However, a major disadvantage of **N5-2OH** as a potential clinical BNCT agent is the lack of water-solubility,[20,21] precipitated by the high hydrophobicity of its *closo-o*-carborane cluster.[22] Several strategies have been developed to improve the water-solubility of 3CTAs including introductions of alcohol-[2,23] and amino functions,[3] PEGylation,[4] and conversion of *closo*-carboranyl 3CTAs to the negatively charged *nido*-carboranyl counterparts.[3]

* To whom correspondence should be addressed. Tel: +1 614 292 7624. Fax: +1 614 292 2435. E-mail: tjarks.1@osu.edu.
[†] Division of Medicinal Chemistry & Pharmacognosy, The Ohio State University.
[‡] Swedish University of Agricultural Sciences.
[§] Department of Pathology, The Ohio State University.
[#] Current address: Department of Cellular and Molecular Medicine, University of Ottawa Heart Institute, Ottawa, Ontario K1Y 4W7, Canada.

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In this paper, novel synthetic approaches to improve TK1 substrate characteristics and bioavailability of 3CTAs are presented. These comprise variations of spacer lengths between carborane cluster and Thd scaffold as well as alterations at the boron cage structure. Particularly, the synthesis and biological evaluation of an extended series of 3CTAs containing a zwitterionic NH_3^+-*nido*-*m*-carborane moiety will be discussed. Recently, two 3CTAs containing this novel carboranyl pharmacophore with unique physicochemical properties have been described by us for the first time.[3] Hitherto only charge-compensated sulfonium *nido*-carboranes were known.[24]

Results and Discussion

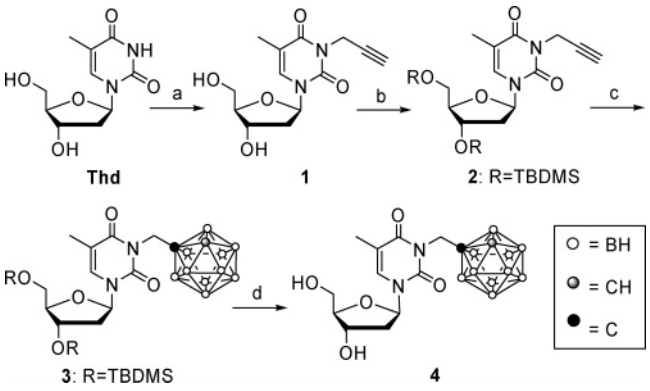
Preliminary studies from our laboratories indicate that 3CTAs presumably are not substrates of nucleoside membrane transporters[25−27] that are sensitive to inhibition by nitrobenzylthioinosine (NBMPR), dilazep, and dipyridamole (D. M. Adams, unpublished results). Also, the main target tumors for BNCT are high grade brain tumors, such as gliomas and astrocytomas,[1] and thus, 3CTAs should have physicochemical properties[28] that facilitate effective penetration of the blood−brain barrier (BBB) and brain tissue. Consequently, 3CTAs should be sufficiently hydrophobic to pass various lipophilic membrane barriers by passive diffusion, while on the other hand, they should also be sufficiently water-soluble for effective administration. **N5-2OH** (Figure 1), which is currently the most promising 3CTA, only fulfills the former criterion.[20,21]

Currently, several strategies are being pursued in our laboratory that may be suitable to circumvent the inherent lack of water-solubility associated with traditional 3CTAs, including encapsulation in emulsion-type nanoparticles, synthesis of water-soluble prodrugs, and optimization of the hydrophilicity/hydrophobicity balance by modulation of functional groups. The latter strategy is the subject of this paper. The water-soluble anti-HIV drug zidovudine (AZT), which is an excellent TK1 substrate[29] that enters cells mainly by passive diffusion,[26] could be considered as a model for this approach and was therefore included as a reference compound in HPLC experiments to assess the relative lipophilicities of the synthesized 3CTAs.

The structural and physicochemical versatility of carborane cages as *closo* and *nido* forms[30,31] was the basis for their selection as target moieties for the optimization of hydrophilicity/hydrophobicity balance of 3CTAs described in this paper. Previously we could demonstrate that simple negatively charged *nido*-carboranyl 3CTAs were substrates of TK1.[3] However, it seems unlikely that such ionic structures can enter cells effectively via passive diffusion. Therefore, 3CTAs with zwitterionic NH_3^+-*nido*-*m*-carborane moieties, exhibiting unique hydrophilicity/hydrophobicity properties, were developed.[3] A detailed description of their synthesis, an extended structure activity relationship (SAR) study, and the preliminary biological evaluation of these compounds are subject of this paper.

We also describe the synthesis of 3-[(*closo*-*o*-carboranyl)methyl]thymidine (**4**). This compound was prepared in order to complete the SAR study of an existing 3CTA library of 3-[(*closo*-*o*-carboranyl)alkyl]thymidines with

Scheme 1[a]



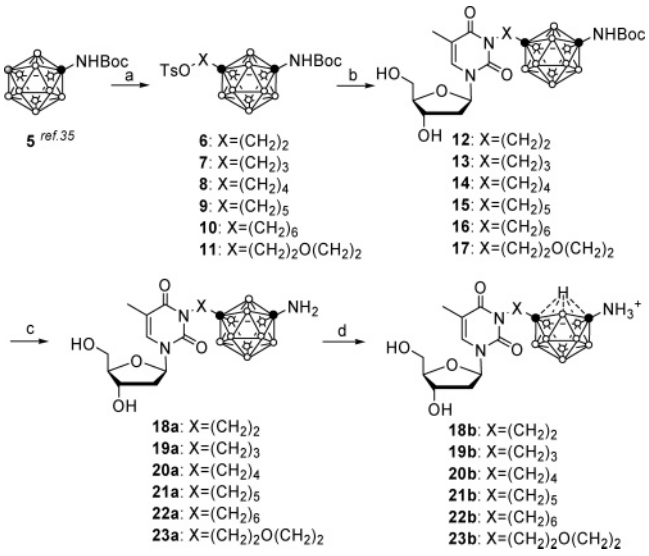
[a] Reagents and conditions: (a) Propargyl bromide, K_2CO_3, DMF/acetone (1:1), 50 °C, 72 h; (b) imidazole, TBDMSCl, DMF, rt, 48 h; (c) $B_{10}H_{14}$, acetonitrile, toluene, reflux, 3 h; (d) TBAF, THF, rt, 30 min.

spacers of 2−7 methylene groups between carborane and Thd scaffold.[2,5] This library was synthesized by alkylation of Thd at the N-3 position using carboranylalkyltosylates. The synthesis of compound **4** (Scheme 1) requires a different strategy since carboranylmethyl halides or tosylates are not suitable for S_N2-type alkylations.[32−34]

Chemistry. The synthetic route for target compound **4** is outlined in Scheme 1. Briefly, the reaction of Thd with propargyl bromide in the presence of K_2CO_3 in DMF/acetone (1:1) at 50 °C for 72 h afforded compound **1** in 61% yield. The 3′- and 5′-hydroxyl groups of compound **1** were silylated using *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF to give compound **2** in 70% yield. Reaction of **2** with a decarborane−acetonitrile complex in toluene under reflux for 3 h provided compound **3** in 25% yield. The relatively low yield may have been caused by steric hindrance by the Thd scaffold during the formation of the bulky carborane cluster. Treatment of **3** with tetrabutylammonium fluoride (TBAF) in THF for 0.5 h gave **4** in 73% yield.

Zwitterionic NH_3^+-*nido*-*m*-carborane-containing 3CTAs were synthesized as shown in Scheme 2. Starting material **5** was prepared on a gram scale applying a procedure previously reported by Kahl et al.[35] The tosylates used for the alkylation of **5** were either purchased [ethylene glycol di-*p*-tosylate, di(ethylene glycol) di-*p*-tosylate] or prepared from 1,3-propanediol, 1,4-butanediol, 1,5-pentanediol, and 1,6-hexanediol as described previously.[36,37] Alkylation of **5** with the alkanediol di-*p*-tosylate and di(ethylene glycol) di-*p*-tosylate using 2.2 mol equiv of n-BuLi in benzene afforded the *m*-carboranyl derivatives **6**−**11** in 14−45% yield. The relatively low yield of **11** (14%) is probably due to both the electron-withdrawing effect of the oxygen atom in the diethylene ether spacer and the low solubility of di(ethylene glycol) di-*p*-tosylate in benzene. Reaction temperature below 10 °C and slow addition of a solution of the lithium salt of **5** in benzene to a solution of alkanediol di-*p*-tosylate in benzene are essential for optimal yields of compounds **6**−**11** and concomitant suppression of the formation of the corresponding dimeric side products. Condensation of compounds **6**−**11** with Thd was carried out in the presence of K_2CO_3 in DMF/acetone (1:1) at 50 °C for 48 h to provide com-

Scheme 2[a]



[a] Reagents and conditions: (a) Alkanediol 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.

pounds **12**−**17** in 50−85% yield. The *tert*-butoxycarbonyl (Boc) group was subsequently removed with trifluoroacetic acid (TFA) in dichloromethane at room temperature for 24 h to give the neutral *closo-m*-carboranyl Thd analogues (**18a**−**23a**) in 69−87% yield. Treatment of **18a**−**23a** with tetrabutylammonium fluoride hydrate (TBAF·xH$_2$O) in THF[38] at 70 °C for 1−2 h and subsequent acidic workup provided the corresponding zwitterionic *nido-m*-carboranyl Thds (**18b**−**23b**) in 50−70% yield. Formation of the *nido*-carboranes was monitored by IR, which showed the disappearance of the typical B−H band of *closo*-carboranes around 2590 cm^{-1} and emergence of the B−H band of *nido*-carboranes around 2530 cm^{-1}.

Analytical C18- and C8 reversed phase HPLC (RP-18 and RP-8) was carried out with compound **1**, **4**, **18a**−**23a**, and **18b**−**23b** (i) for purity verification (Supporting Information Available), (ii) to provide 1−3 mg highly purified quantities for phosphoryl transfer assays (PTAs) (Figure 2 and Table 1), and (iii) to obtain retention times as measures of their lipophilicities (Table 1). All compounds were analyzed by ^{1}H NMR, ^{13}C NMR, and HRMS.

In the case of HPLC analysis of compound **18b**, two close peaks were observed at 14.21 and 14.43 min (RP-18) as well 14.49 and 14.65 min (RP-8). A splitting pattern was also observed for several signals in the ^{1}H NMR and ^{13}C NMR spectra of compound **18b**, in particular for the C-1′ proton (6.27 and 6.36 ppm). Similar splitting patterns were not observed for **19b**−**23b** and **18a**−**23a**. Degradation of *closo-m*-carborane compounds into its *nido-m*-counterpart introduces a chiral element, which in the case of the zwitterionic 3CTAs **18b**−**23b** results a mixture of two diastereomers. The close proximity of the bulky *nido*-carborane cluster to the Thd scaffold in **18b** could cause additional steric constraint emphasizing structural differences between both diastereomers and thereby leading to the observed splitting patterns both in HPLC and NMR spectra. However, splitting of some signals was also observed

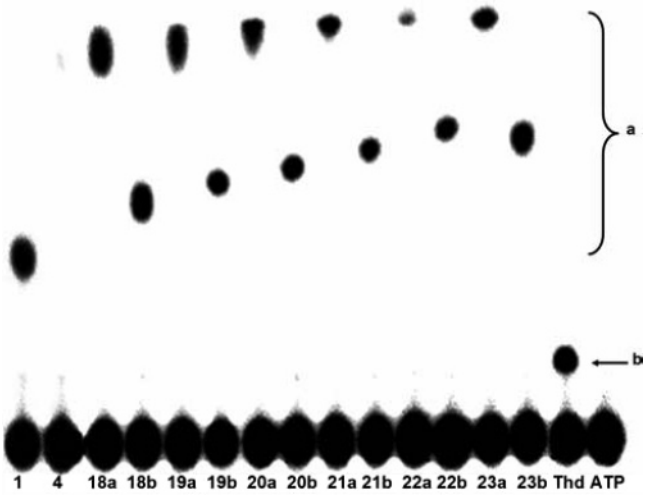


Figure 2. Phosphorylation of 3CTAs and Thd by recombinant TK1 (see Experimental Section for details). The reaction products were separated by PEI-cellulose TLC and visualized by ^{32}P-autoradiograhy. (a) Monophosphate products of the target compounds **1**, **4**, and **18a/b**−**23a/b**. (b) Thd-5′-monophosphate.

Table 1. TK1/TK2 Phosphorylation Rates and RP-18 Retention Times of 3CTAs, Thd, and AZT

compd	TK1 (10 μM)	TK2 (100 μM)	RP-18 retention times
1	102.1 ± 3.6	5.0	11.79
4	9.6 ± 2.4	<0.1	22.71
18a	71.9 ± 5.1	<0.1	19.01
18b	89.4 ± 1.2	<0.1	14.32[a]
19a	58.2 ± 2.5	<0.1	19.08
19b	64.6 ± 4.0	<0.1	14.96
20a	58.0 ± 5.3	0.4	20.81
20b	63.8 ± 3.1	0.1	15.38
21a	45.1 ± 2.6	0.4	22.65
21b	50.7 ± 1.6	1.2	16.36
22a	14.2 ± 2.1	0.7	24.76
22b	45.4 ± 2.0	1.3	17.77
23a	48.4 ± 1.6	0.9	18.08
23b	75.1 ± 0.6	0.5	14.48
N5-2OH[b]	41.0 ± 5.0	<0.1	22.54
AZT[c]	52.0	4.0	12.22
Thd[d]	100.0	100.0[d]	8.72

[a] Two peaks were observed both in analytical C18- and C8 reversed phase HPLC. The average value of both peaks (14.21 and 14.43 min) was inserted. [b,c] TK1 and TK2 phosphorylation rates were taken from ref 2[b] and 41[c], respectively. [d] Phosphorylation rates for Thd were set to 100. Mean ± SD values are based on three experiments for recombinant TK1 and one experiment for TK2.

in the ^{13}C NMR spectra of the *closo-o*-carboranes compounds **3** and **4**, although not to same extent and the HPLC chromatogram of **4** did not show any evidence of isomeric mixtures. This indicates the formation of atropoisomers in the case of compounds **3** and **4** due to the hindered rotation of single bonds between carborane cluster and Thd scaffold. Therefore, it is also conceivable that the splitting patterns observed in case of **18b** are solely based on the formation of atropoisomers as well. An in-depth investigation of the unusual structural and physicochemical properties of target compounds **18a/b**−**23a/b** including pK_a, log P, Clog P measurements and calculation as well as X-ray crystallographic studies is currently underway. Such data are, however, beyond the scope of this paper and will be published elsewhere.

Biology. The β-autoradiogram of the monophosphate (MP) products of compounds **1**, **4**, and **18a/b**−**23a/b** ('**a**')

as well as Thd-MP ('**b**') is shown in Figure 2. It was developed from a PEI-cellulose TLC plate on which the reaction products of the PTAs of **Thd**, **1**, **4**, and **18a/b**−**23a/b** were separated (see Experimental Section). In previous studies with similar 3CTAs, combined α/β-autoradiography provided evidence that the observed intense spots with high R_f values ('**a**') are typical for MPs of 3CTAs.[2,5,39] The exact relative phosphorylation rates of compounds **1**, **4**, and **18a/b**−**23a/b** with TK1 and the mitochondrial isozyme thymidine kinase 2 (TK2) are summarized in Table 1. Compound **4**, with a methylene spacer between carborane cluster and Thd scaffold, showed a TK1 phosphorylation rate of 9.6 ± 2.4%, which is in the same range of those of its butylene (13%) and heptylene (11%) homologues but lower than those of its ethylene (39%), propylene (30%), pentylene (41%), and hexylene (28%) homologues in a library of the previously reported 3CTAs.[2] This indicates that a carborane cluster in close proximity to the N-3 position of Thd may have a negative impact on the phosphorylation rates of 3CTAs. Indeed, only 3-[(*closo-o*-carboranyl)alkyl]thymidines with ethylene and pentylene spacers consistently have shown superior TK1 substrate/inhibitor characteristics and overall biological properties compared with those having propylene, butylene, hexylene, and heptylene spacers.[2,20,21] Noteworthy are the TK1 (102.1 ± 3.6%) and the TK2 (∼5%) phosphorylation rates of 3-(2-propyn-1-yl)thymidine (**1**), indicating that both TK enzymes tolerate relatively small substituents at the N-3 position of Thd, although to a substantially different degree.

The TK1 phosphorylation rates of compounds **18a**−**23a** (neutral *closo* series) ranged from 71.9 ± 5.1% to 14.2 ± 2.1% and those of compounds **18b**−**23b** (zwitterionic *nido* series) ranged from 89.4 ± 1.2% to 45.4 ± 2.0% (Table 1). The TK1 phosphorylation rate of **18b** (89.4 ± 1.2%) is the highest reported for any 3CTA. With the exception of compounds **23a/b**, having diethylene ether spacers, the TK1 phosphorylation rates decreased in both series with increasing spacer length almost in a linear fashion and those of the neutral *closo-m*-carboranyl series **18a**−**23a** were 5−30% lower than those of the zwitterionic *nido-m*-carboranyl series **18b**−**23b**.

There is a very characteristic pattern in the differences of PEI-TLC R_f values between the MPs of the neutral *closo-m*-carboranyl series **18a**−**23a** and the MPs of the zwitterionic *nido-m*-carboranyl series **18b**−**23b**, as shown in the autoradiogram in Figure 2, which corresponds to some extent with the pattern of the RP-18 retention times of nonphosphorylated **18a**−**23a** and **18b**−**23b** depicted in Figure 3. As shown in Figure 3, the TK1 phosphorylation rates of **18a/b**−**23a/b** are almost inversely proportional to their RP-18 retention time, and thus, to their lipophilicity. In the cases of zwitterionic compound **23b**, containing a diethylene ether spacer, the RP-18 retention time and the phosphorylation rate are overall more comparable with those of compounds **18b**−**20b**, having ethylene, propylene, and butylene spacers rather than those of **21b**−**22b** with pentylene and hexylene spacers comparable in lengths to the diethylene ether spacer in **23b**. Therefore, it appears that the degree of lipophilicity of **18b**−**23b** rather than the spacer lengths of these structures is the determining factor for their TK1 phosphorylation rates. However, we cannot exclude the possibility that the oxygen atom in the spacer of **23b** affects the TK1 phosphorylation rate of these compounds through specific hydrogen acceptor interactions in the substrate binding site of TK1 and that the spacer length is therefore also a critical factor. In any case, the SAR of **18a/b**−**23a/b** appears to follow a completely different pattern than the SARs of different types of 3CTAs that were previously evaluated.[2,4,21] The three-dimensional structure of human TK1 recently has been determined by X-ray crystallography.[40] This structure may facilitate the detailed analysis of the SARs of various types of 3CTAs. Compound **18b** had a significantly higher TK1 phosphorylation rate than AZT (52%),[41] while its RP-18 retention time was slightly longer (∼2 min). Thus, **18b** should be sufficiently lipophilic for passive diffusion through cell membranes despite the zwitterionic nature of its carboranyl moiety.

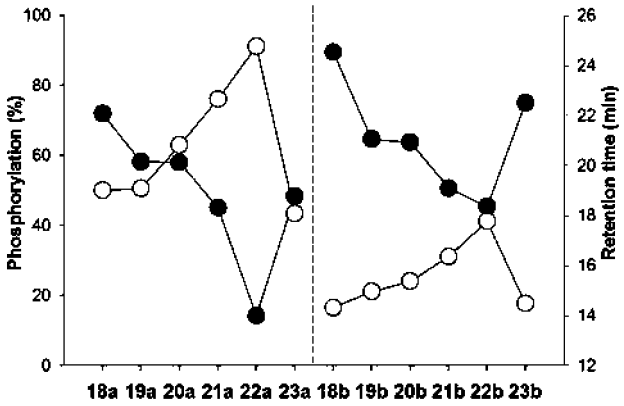


Figure 3. Correlation between TK1 phosphorylation and RP-18 retention times of compounds **18a/b**−**23a/b**. ● = TK1 phosphorylation of the neutral *closo-m*-carboranyl series **18a**−**23a** and the zwitterionic *nido-m*-carboranyl series **18b**−**23b**. ○ = RP-18 retention times of compounds **18a/b**−**23a/b**.

Only at high substrate concentrations of 100 μM, compounds **20a/b**−**23a/b**, having butylene, pentylene, hexylene, and diethylene ether spacers, appeared to be phosphorylated to a limited extent by TK2 with phosphorylation rates ranging from 0.1 to 1.3%, while **18a/b**−**19a/b** with shorter ethylene- and propylene spacers apparently were not substrates for mitochondrial TK2. In general, 3CTAs that are effective substrates of TK2 are probably not suitable as BNCT agents because this kinase is equally active in both normal and proliferating cells.[41]

The zwiterionic compound **19b** was selected for preliminary in vivo biodistribution studies in mice bearing subcutaneous (sc) L929 (wt) or L929 TK1 (−) tumors. At the time of this experiment, compound **18b**, which has a higher TK1 phosphorylation rate than **19b**, had not yet been synthesized. BPA, which is currently used in clinical BNCT trials in the US, Japan, Argentina, and Europe,[42−47] and **N5-2OH** were used as reference compounds.[20] Since brain tumors are the preferred target of BNCT,[42,43] **N5-2OH** previously has been evaluated in two different rodent/brain tumor models using intracerebral (i.c.) convection-enhanced delivery (CED) as the route of administration.[20] In this procedure, **N5-2OH** was injected directly into the brain using the same stereo tactic coordinates that were used for the implan-

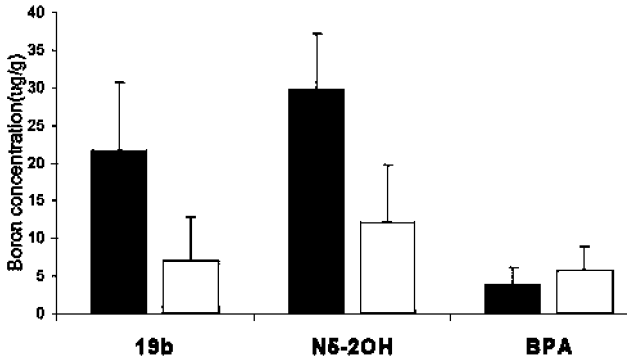


Figure 4. Tumor uptake of **19b**, **N5-2OH**, and BPA in mice bearing sc implants of L929 (wt) and L929 TK1 (−) tumor. Compound quantities equivalent to a total of 100 μg of boron were given in two intratumoral injections in a 2 h interval. Boron concentrations in tissues were determined 2 h following the final injection by DCP-AES.[51] Each data point represents the arithmetic mean ± SD of four mice. ■: L929 (wt) tumors, □: L929 TK1 (−) tumors.

tation of the cancer cells into the rodent brains for tumor growth. The advantages of CED are, for example, preferential exposure of target tissue to high **N5-2OH** concentrations, circumventing the BBB, and reduced systemic toxicity.[20] In the preliminary in vivo studies described here, sc tumors were used since their implantation was easier than those of ic tumors. All compounds were injected twice intratumorally (it) with a 2 h-interval since this route mimics to some extent the ic. CED. Boron concentrations in L929 (wt) tumors were 21.7 ± 9.1, 29.8 ± 7.4,[20] and 4.0 ± 2.1[20] μg/g tumor for **19b**, **N5-2OH**, and BPA (Figure 1), respectively, at 2 h following the 2nd injection of compound quantities each equivalent to 50 μg boron (Figure 4). The corresponding values for the L929 TK1 (−) tumors, were 7.0 ± 5.9, 12.1 ± 7.7,[20] and 5.8 ± 3.1[20] μg/g tumor. Boron concentrations in blood, skin, and liver were <0.5 μg/g tissue in both tumor/rodent models. The values for **19b** and **N5-2OH** in L929 (wt) tumors were comparable and in the concentration range necessary for BNCT, while those for BPA were significantly lower. The values for nucleoside analogues **19b** and **N5-2OH** in L929 TK1 (−) tumors were 3.1 and 2.5 times lower than in L929 (wt) tumors while that of the amino acid BPA was increased by a factor of 1.5. These results indicated that the uptake of **19b** and **N5-2OH** is related to some form of nucleoside metabolism, presumably intracellular entrapment through 5′-monophosphorylation. However, we cannot exclude the possibility that cellular efflux mechanisms specific to L929 TK1 (wt) and L929 TK1 (−) maybe contribute to the observed biodistribution patterns. To dissolve compound quantities equivalent to 50 μg of boron, 15 μL of DMSO/H_2O (70:30) was required for **N5-2OH** while in case of **19b**, 10 μL of DMSO/H_2O (24:76) was sufficient, indicating significantly improved water solubility properties of **19b**.

Summary and Conclusions

The six zwitterionic NH_3^+-*nido-m*-carborane-containing 3CTAs and their corresponding neutral NH_2-*closo-m*-carborane-substituted counterparts were synthesized. Their phosphorylation rates were evaluated in PTAs with purified recombinant human TK1 and TK2. The TK1 phosphorylation rates ranged from 89% to 14% in this series of 12 target compounds and appeared to be dependent mainly on the lipophilicity of the compounds although structural contribution, such as the spacer length between carborane cluster and Thd scaffold, may also be of importance. The TK1 phosphorylation rate of zwitterionic compound **18b** (89%) was the highest determined for any 3CTA. HPLC RP-18 retention times indicated that the lipophilicity of zwitterionic compounds such as **18b** and **19b** may be sufficient for passive diffusion through cell membranes. Compounds of this series containing butylene, pentylene, hexylene, and diethylene ether spacers also appeared to be phosphorylated to a limited extent by TK2. Compound **4**, which differs structurally from **18a/b**−**23a/b**, did not have sufficient TK1 substrate activity. Compound **19b** was preferentially taken up in L929 (wt) tumors implanted in nude mice and it had significantly improved water solubility compared with **N5-2OH**. Overall, the class of zwitterionic NH_3^+-*nido-m*-carborane-containing 3CTAs described in this paper appears to have similar or even improved biological properties than those of other classes of 3CTAs described previously[2,4,20,21] combined with significantly improved physicochemical properties. An in-depth evaluation of their structural, physicochemical, and biological properties including X-ray crystallographic studies, pK_a, log P, and Clog P measurements and calculations, detailed enzymatic analyses (K_i and k_{cat}/K_m values), as well as in vitro and extended in vivo studies are underway to facilitate the further development of this promising compound class into boron delivery agents for BNCT.

Experimental Section

^{1}H NMR, ^{11}B NMR, and ^{13}C NMR spectra were obtained on Bruker (250 or 400 MHz) FT-NMR instruments. Chemical shifts are reported in parts per million (ppm) from an internal tetramethylsilane standard. The coupling constants are reported in Hertz (Hz). High-resolution electrospray ionization mass spectra (HR-ESI) were recorded on a Micromass QTOF-Electrospray mass spectrometer and a 3-Tesla Finnigan FTMS-2000 Fourier Transform mass spectrometer at The Ohio State University Campus Chemical Instrumentation Center (OSU-CCIC) and The Ohio State University Department of Chemistry by members of these institutions including Dr. Kari Green-Church, Robin Gates, and Professor Christopher M. Hadad. Compound visualization on Silica Gel 60 F_{254}-precoated TLC plates (0.25 mm layer thickness) (Merck, Darmstadt, Germany) was attained by UV light and $KMnO_4$ spray. Carborane-containing compounds were selectively visualized by spraying a solution of 0.06% $PdCl_2$/1% aqueous HCl on TLC plates and subsequent heating to ∼120 °C, which caused the slow formation (15−45 s) of gray spots due to the reduction of Pd^{2+} to Pd^0. Reagent grade solvents were used for column chromatography using Silica gel 60, particle size 0.040−0.063 mm (Merck, Rahway, NJ). Analytical HPLC data of the target compounds were obtained with reversed phase C8 (RP-8) and C18 (RP-18) LiChrosphere 100 Å [5 μm] columns (Merck,) using a Rainin HPLC instrument equipped with a Dynamax DA controller, HPXL pumps, and a Dynamax UV-1 detector (Rainin Instrument Company Inc., Woburn, MA). HPLC grade water and methanol were used as solvents. A water/methanol gradient (100:0 to 70:30 over 10 min, from 70:30 to 90:10 over 20 min, and from 90:10 to 0:100 over 10 min) with a flow rate of 1 mL/min was applied for all target compounds. Reagent grade chemicals were obtained from commercial vendors and used as such. Solvents were dried prior to use following standard procedures. All reactions were carried out under argon atmosphere.

3-(2-Propyn-1-yl)thymidine (1). A solution of propargyl bromide (1.5 mL, 13.47 mmol) was added to a solution of

thymidine (2.45 g, 10.03 mmol) and potassium carbonate (3.80 g, 27.49 mmol) in DMF/acetone (50 mL, 1:1) and stirred at 50 °C for 72 h. The reaction mixture was filtered and evaporated to dryness. Distilled water (20 mL) was added to the residue, and the solution was extracted with dichloromethane (3 × 20 mL). The organic layers were combined and washed with brine and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (chloroform/acetone, 1:1) to give compound **1** as a white solid in 61% (1.72 g) yield. ^{1}H NMR (D_2O): δ 1.77 (s, 3H, CH_3), 2.13−2.23 (m, 2H, H-2′), 2.45 (s, 1H, CH≡C), 3.39−3.60 (m, 2H, H-5′), 3.73 (m, 1H, H-3′), 4.32 (m, 1H, H-4′), 4.51 (s, 2H, CH_2N), 6.14 (t, 1H, H-1′, J = 6.4 Hz), 7.54 (s, 1H, H-6); ^{13}C NMR (D_2O): δ 12.16 (CH_3), 30.67 (CH_2N), 38.79 (C-2′), 61.07 (C-5′), 70.36 (C-3′), 71.79 (C≡*C*H), 77.92 (*C*≡CH), 85.95 (C-1′), 86.56 (C-4′), 110.56 (C-5), 135.82 (C-6), 150.88 (C-2), 164.41 (C-4); MS (HR-ESI) $C_{13}H_{16}N_2O_5Na$ $(M + Na)^+$ calcd: 303.0957, found: 303.0966; RP-18: 11.79 min, RP-8: 11.99 min, >98% pure.

[4′,5′-Di(*tert*-butyldimethylsilanyl)-3-(2-propyn-1-yl)]-thymidine (2). A solution of *tert*-butyldimethylsilyl chloride (2.04 g, 13.50 mmol) in DMF (4 mL) was added to a solution of compound **1** (1.72 g, 6.14 mmol) and imidazole (1.46 g, 21.48 mmol) in DMF (6 mL) at 0 °C, stirred for 20 min at the same temperature, and then for 48 h at room temperature. After evaporation, the residue was extracted with dichloromethane (50 mL) and washed with water (5 × 30 mL). The organic layer was washed with brine and dried over sodium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (hexanes/ethyl acetate, 6:1) to give compound **2** as colorless oil in 70% (2.20 g) yield. ^{1}H NMR ($CDCl_3$): δ 0.03 (s, 6H, CH_3Si), 0.07 (s, 6H, CH_3Si), 0.82 (s, 9H, $C(CH_3)_3$), 0.84 (s, 9H, $C(CH_3)_3$), 1.88 (d, 3H, CH_3, J = 1.0 Hz), 2.11 (s, 1H, CH≡C), 1.98−2.28 (m, 2H, H-2′), 3.72−3.95 (m, 3H, H-5′ and H-3′), 4.38 (m, 1H, H-4′), 4.62 (s, 2H, CH_2N), 6.31 (t, 1H, H-1′, J = 6.2 Hz), 7.44 (d, 1H, H-6, J = 1.0 Hz); ^{13}C NMR ($CDCl_3$): δ −5.85 (CH_3Si), −5.78 (CH_3Si), −5.69 (CH_3Si), −5.05 (CH_3Si), 12.76 (CH_3), 17.43 ($C(CH_3)_3$), 17.54 ($C(CH_3)_3$), 25.34 (CH_3), 25.53 (CH_3), 29.86 (CH_2N), 41.00 (C-2′), 62.59 (C-5′), 70.09 (C-3′), 71.90 (C≡*C*H), 77.92 (*C*≡CH), 85.17 (C-1′), 87.38 (C-4′), 109.49 (C-5), 133.42 (C-6), 149.71 (C-2), 161.91 (C-4); MS (HR-ESI) $C_{25}H_{44}N_2O_5Si_2Na$ $(M + Na)^+$ calcd: 531.2686, found: 531.2691.

{4′,5′-Di(*tert*-butyldimethylsilanyl)-3-[(*closo*-*o*-caboran-1-yl)methyl]}thymidine (3). A solution of decaborane (700 mg, 4.09 mmol) in toluene/acetonitrile (22 mL, 10:1) was refluxed for 1 h. (*Caution: Decaborane is a highly toxic, impact sensitive compound, which forms explosive mixtures especially with halogenated materials. A careful study of the MSDS is advisable before usage*). The solution was cooled to room temperature, a solution of compound **2** (500 mg, 0.99 mmol) in toluene (30 mL) was added, and the reaction mixture was stirred under reflux for 3 h. The solvent was evaporated and the residue suspended in diethyl ether, filtered off, and washed with the same solvent. The filtrate was evaporated, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate, 8:1) to give compound **3** in 25% (160 mg) yield. ^{1}H NMR ($CDCl_3$): δ 0.08 (s, 12H, CH_3Si), 0.90 (s, 18H, $C(CH_3)_3$), 1.91 (d, 3H, CH_3, J = 1.1 Hz), 1.92−2.28 (m, 2H, H-2′), 3.71−3.96 (m, 3H, H-5′ and H-3′), 4.18 (br s, 1H, *H*-$C_{carborane}$), 4.32 (m, 1H, H-4′), 4.57−4.71 (m, 2H, CH_2N), 6.29 (t, 1H, H-1′, J = 6.7 Hz), 7.53−7.55 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR ($CDCl_3$): δ −5.25 (CH_3Si), −5.20 (CH_3Si), −4.66 (CH_3Si), −4.50 (CH_3Si), 13.35 (CH_3), 18.15 ($C(CH_3)_3$), 18.19 ($C(CH_3)_3$), 25.81 (CH_3), 25.89 (CH_3), 41.79 (C-2′), 45.38 (CH_2N), 63.21 (C-5′), 72.56 (C-3′), 86.26 (C-1′), 88.43 (C-4′), 109.99 (C-5), 134.83 (C-6), 150.71 (C-2), 163.14 (C-4); MS (HR-ESI) $C_{25}H_{54}B_{10}N_2O_5Si_2Na$ $(M + Na)^+$ calcd: 651.4399, found: 651.4387.

3-[(*closo*-*o*-Caboran-1-yl)methyl]thymidine (4). A solution of tetrabutylammonium fluoride in THF (1.0 M, 0.40 mL, 0.40 m mol) was added to a solution of compound **3** (120 mg, 0.19 mmol) in THF (3 mL) at 0 °C. The mixture was stirred for 30 min at room temperature and evaporated. The residue was coevaporated with anhydrous triethylamine (1.5 mL). The residue was dissolved in ethyl acetate (30 mL) and washed with diluted HCl (3%, 15 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated, and the residue was purified by silica gel column chromatography (dichloromethane/acetone, 2:1) to give compound **4** in 73% (55 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.89 (d, 3H, CH_3, J = 1.1 Hz), 2.20−2.35 (m, 2H, H-2′), 3.78−3.99 (m, 3H, H-5′ and H-3′), 4.11 (m, 1H, H-4′), 4.50−4.55 (m, 2H, CH_2N), 6.16 (t, 1H, H-1′, J = 6.7 Hz), 7.56 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR ($CDCl_3$): δ 13.21 (CH_3), 40.42 (C-2′), 45.20 (CH_2N), 61.46 (C-5′), 62.22 ($C_{carborane}$-C), 71.45 (C-3′), 73.23 ($C_{carborane}$-C), 86.92 (C-1′), 87.22 (C-4′), 110.16 (C-5), 135.92 (C-6), 150.66 (C-2), 163.05 (C-4); MS (HR-ESI) $C_{13}H_{26}B_{10}N_2O_5Na$ $(M + Na)^+$ calcd: 421.2750, found: 421.2759; RP-18: 22.71 min, RP-8: 21.37 min, >98% pure.

2-{[7-(*tert*-Butyloxy)carbonylamino]-*closo*-*m*-carboran-1-yl}ethyl Tosylate (6). Butyllithium in hexanes (2.5 M, 1.53 mL, 3.83 mmol) was slowly added to a solution of compound **5**[35] (450 mg, 1.74 mmol) in benzene (15 mL) at 5−10 °C and stirred for 20 min at the same temperature. The mixture was warmed to room temperature and stirred for additional 30 min. This solution of the lithium salt of compound **6** in benzene was added very slowly to a solution of ethylene glycol di-*p*-tosylate (1.6 g, 4.32 mmol) in benzene (5 mL) at 5−10 °C. The mixture was stirred for 30 min at the same temperature, distilled water (20 mL) was added, and benzene was removed under reduced pressure. The residue was extracted with ethyl acetate (3 × 15 mL), and the combined organic layers were washed with brine and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (hexanes/ethyl acetate, 12:1) to give compound **6** as colorless oil in 35% (280 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H, $C(CH_3)_3$), 2.29 (t, 2H, CH_2-$C_{carborane}$, J = 7.1 Hz), 2.44 (s, 3H, CH_3), 3.94 (t, 2H, CH_2O, J = 7.1 Hz), 5.07 (s, 1H, NH), 7.34 (d, 2H, Ar−H, J = 8.3 Hz), 7.76 (m, 2H, Ar−H, J = 8.3 Hz);^{13}C NMR ($CDCl_3$): δ 21.69 (CH_3), 28.13 (CH_3), 33.47 (CH_2-$C_{carborane}$), 67.35 ($OC(CH_3)_3$), 69.37 (CH_2O), 80.72 ($C_{carborane}$-C), 82.89 ($C_{carborane}$-C), 127.93 (Ar-C), 129.98 (Ar-C), 132.97 (Ar-C), 145.05 (Ar-C), 152.04 (C=O); MS (HR-ESI) $C_{16}H_{31}B_{10}NO_5SNa$ $(M + Na)^+$ calcd: 480.2833, found: 480.2802.

3-{[7-(*tert*-Butyloxy)carbonylamino]-*closo*-*m*-carboran-1-yl}propyl Tosylate (7). Compound **7** was prepared according to the procedure described for compound **6** using butyllithium in hexane (2.5 M, 1.04 mL, 2.60 mmol), compound **5** (307 mg, 1.18 mmol), and 1,3-propanediol di-*p*-tosylate (1.00 g, 2.6 mmol). Purification by silica gel column chromatography (hexanes/ethyl acetate, 12:1) gave **7** as colorless oil in 36% (200 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H, $C(CH_3)_3$), 1.63−1.67 (m, 2H, CH_2), 1.90−1.97 (m, 2H, CH_2-$C_{carborane}$), 2.43 (s, 3H, CH_3), 3.91 (t, 2H, CH_2O, J = 7.0 Hz), 5.13 (s, 1H, NH), 7.33 (d, 2H, Ar−H, J = 8.1 Hz), 7.73 (d, 2H, Ar−H, J = 8.1 Hz);^{13}C NMR ($CDCl_3$): δ 21.65 (CH_3), 28.14 (CH_3), 28.99 (CH_2), 33.03 (CH_2-$C_{carborane}$), 69.01 ($OC(CH_3)_3$), 72.37 (CH_2O), 80.72 ($C_{carborane}$-C), 81.70 ($C_{carborane}$-C), 127.89 (Ar-C), 129.96 (Ar-C), 132.78 (Ar-C), 145.04 (Ar-C), 151.85 (C=O); MS (HR-ESI) $C_{17}H_{33}B_{10}NO_5SNa$ $(M + Na)^+$ calcd: 494.2975, found: 494.2997.

4-{[7-(*tert*-Butyloxy)carbonylamino]-*closo*-*m*-carboran-1-yl}butyl Tosylate (8). Compound **8** was prepared according to the procedure described for compound **6** using butyllithium in hexanes (2.5 M, 1.00 mL, 2.50 mmol), compound **5** (258 mg, 1.00 mmol), and 1,4-butanediol di-*p*-tosylate (600 mg, 1.51 mmol). Purification by silica gel column chromatography (hexanes/ethyl acetate, 14:1) gave **8** as colorless oil in 43% (210 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.25−1.35 (m, 4H, CH_2), 1.41 (s, 9H, $C(CH_3)_3$), 1.49−1.52 (m, 2H, CH_2), 1.81−1.88 (m, 2H, CH_2-$C_{carborane}$), 2.44 (s, 3H, CH_3), 3.95 (t, 2H, CH_2O, J = 6.2 Hz), 5.09 (s, 1H, NH), 7.34 (d, 2H, Ar−H, J = 8.0 Hz), 7.77 (d, 2H, Ar−H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$): δ 21.64 (CH_3), 25.81 (CH_2), 28.12 (CH_3), 28.25 (CH_2), 36.31 (CH_2-$C_{carborane}$), 69.65 ($OC(CH_3)_3$), 73.21 (CH_2O), 80.51 ($C_{carborane}$-C), 81.63 ($C_{carborane}$-C), 127.86 (Ar-C), 129.93 (Ar-C), 132.87 (Ar-C), 144.89 (Ar-C), 151.86 (C=O); MS (HR-ESI) $C_{18}H_{35}B_{10}NO_5SNa$ $(M + Na)^+$ calcd: 508.3147, found: 508.3169.

5-{[7-(*tert*-Butyloxy)carbonylamino]-*closo-m*-carboran-1-yl}pentyl Tosylate (9). Compound **9** was prepared according to the procedure described for compound **6** using butyllithium in hexanes (2.5 M, 1.82 mL, 4.56 mmol), compound **5** (560 mg, 2.17 mmol), and 1,5-pentanediol di-*p*-tosylate (983 mg, 2.39 mmol). Purification by silica gel column chromatography (hexanes/ethyl acetate, 14:1) gave **9** as colorless oil in 39% (420 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.20−1.28 (m, 4H, CH_2), 1.42 (s, 9H, $C(CH_3)_3$), 1.57−1.62 (m, 2H, CH_2), 1.83−1.89 (m, 2H, CH_2-$C_{carborane}$), 2.45 (s, 3H, CH_3), 3.98 (t, 2H, CH_2O, J = 6.2 Hz), 5.12 (s, 1H, NH), 7.34 (d, 2H, Ar−H, J = 7.9 Hz), 7.78 (d, 2H, Ar−H, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$): δ 21.63 (CH_3), 24.88 (CH_2), 28.12 (CH_3), 28.35 (CH_2), 29.10 (CH_2), 36.76 (CH_2-$C_{carborane}$), 70.03 ($OC(CH_3)_3$), 73.60 (CH_2O), 80.52 ($C_{carborane}$-C), 81.59 ($C_{carborane}$-C), 127.84 (Ar-C), 129.85 (Ar-C), 133.02 (Ar-C), 144.79 (Ar-C), 151.89 (C=O); MS (HR-ESI) $C_{19}H_{37}B_{10}NO_5SNa$ $(M + Na)^+$ calcd: 522.3288, found: 522.3263.

6-{[7-(*tert*-Butyloxy)carbonylamino]-*closo-m*-carboran-1-yl}hexyl Tosylate (10). Compound **10** was prepared according to the procedure described for compound **6** using butyllithium in hexanes (2.5 M, 1.00 mL, 2.50 mmol), compound **5** (258 mg, 1.00 mmol), and 1,6-hexanediol di-*p*-tosylate (650 mg, 1.52 mmol). Purification by silica gel column chromatography (hexanes/ethyl acetate, 15:1) gave **10** as colorless oil in 45% (230 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.10−1.12 (m, 2H, CH_2), 1.21−1.27 (m, 4H, CH_2), 1.40 (s, 9H, $C(CH_3)_3$), 1.55−1.59 (m, 2H, CH_2), 1.82−1.86 (m, 2H, CH_2-$C_{carborane}$), 2.43 (s, 3H, CH_3), 3.97 (t, 2H, CH_2O, J = 6.4 Hz), 5.11 (s, 1H, NH), 7.32 (d, 2H, Ar−H, J = 8.0 Hz), 7.74 (d, 2H, Ar−H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$): δ 21.63 (CH_3), 24.95 (CH_2), 28.12 (CH_3), 28.36 (CH_2), 28.58 (CH_2), 29.62 (CH_2), 36.88 (CH_2-$C_{carborane}$), 70.31 ($OC(CH_3)_3$), 73.85 (CH_2O), 80.49 ($C_{carborane}$-C), 81.57 ($C_{carborane}$-C), 127.85 (Ar-C), 129.81(Ar-C), 133.08 (Ar-C), 144.70 (Ar-C), 151.89 (C=O); MS (HR-ESI) $C_{20}H_{39}B_{10}NO_5SNa$ $(M + Na)^+$ calcd: 536.3461, found: 536.3467.

2-{2-[7-(*tert*-Butyloxy)carbonylamino]-*closo-m*-carboran-1-yl}ethyloxyethyl Tosylate (11). Compound **11** was prepared according to the procedure described for compound **6** using butyllithium in hexanes (2.5 M, 2.04 mL, 5.10 mmol), compound **5** (600 mg, 2.32 mmol), and di(ethylene glycol) di-*p*-tosylate (1.05 g, 2.55 mmol). Purification by silica gel column chromatography (hexanes/ethyl acetate, 5:1) gave **11** as colorless oil in 14% (160 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H, $C(CH_3)_3$), 2.09 (t, 2H, CH_2-$C_{carborane}$, J = 6.8 Hz), 2.44 (s, 3H, CH_3), 3.30 (t, 2H, CH_2O, J = 6.8 Hz), 3.53 (t, 2H, CH_2O, J = 4.5 Hz), 4.11 (t, 2H, CH_2O, J = 4.5 Hz), 5.13 (br s, 1H, NH), 7.32 (d, 2H, Ar−H, J = 7.9 Hz), 7.77 (d, 2H, Ar−H, J = 7.9 Hz); MS (HR-ESI) $C_{18}H_{35}B_{10}NO_6SNa$ $(M + Na)^+$ calcd: 524.3086, found: 524.3257.

3-{2-[(7-(*tert*-Butyloxy)carbonylamino)-*closo-m*-carboran-1-yl]ethan-1-yl}thymidine (12). A solution of compound **6** (270 mg, 0.59 mmol), thymidine (440 mg, 1.82 mmol), and potassium carbonate (450 mg, 3.26 mmol) in DMF/acetone (20 mL, 1:1) was stirred at 50 °C for 48 h. The solution was filtered and evaporated. The residue was dissolved in the water (20 mL) and extracted with ethyl acetate (4 × 20 mL). The combined organic layers were washed with brine and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (ethyl acetate/methanol, 15:1) to give compound **12** as a white solid in 64% (200 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 9H, $C(CH_3)_3$), 1.86 (s, 3H, CH_3), 2.15−2.20 (m, 2H, H-2′), 2.27−2.32 (t, 2H, CH_2-$C_{carborane}$, J = 6.3 Hz), 3.82−3.99 (m, 5H, H-3′, H-5′ and CH_2N), 4.51−4.55 (m, 1H, H-4′), 5.34 (s, 1H, NH), 6.17 (t, 1H, H-1′, J = 6.7 Hz), 7.43 (s, 1H, H-6);^{13}C NMR (MeOH-d_4): δ 13.09 (CH_3), 28.51 (CH_3), 34.21 (CH_2-$C_{carborane}$), 41.31 (CH_2N), 41.39 (C-2′), 62.69 (C-5′), 71.45 (C-3′), 72.01 ($OC(CH_3)_3$), 81.66 ($C_{carborane}$-C), 83.21 ($C_{carborane}$-C), 87.12 (C-1′), 88.85 (C-4′), 110.60 (C-5), 136.65 (C-6), 151.86 (C=O), 154.58 (C=O), 164.92 (C=O); MS (HR-ESI) $C_{19}H_{37}B_{10}N_3O_7Na$ $(M + Na)^+$ calcd: 550.3542, found: 550.3578.

3-{3-[(7-(*tert*-Butyloxy)carbonylamino)-*closo-m*-carboran-1-yl]propan-1-yl}thymidine (13). Compound **13** was prepared according to the procedure described for compound **12** using compound **7** (170 mg, 0.36 mmol), thymidine (104 mg, 0.43 mmol), and potassium carbonate (150 mg, 1.08 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **13** as a white solid in 85% (166 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H, $C(CH_3)_3$), 1.56−1.67 (m, 2H, CH_2), 1.90 (s, 3H, CH_3), 1.90−2.02 (m, 2H, H-2′), 2.30−2.38 (m, 2H, CH_2-$C_{carborane}$), 3.80−3.98 (m, 5H, H-3′, H-5′ and CH_2N), 4.56−4.59 (m, 1H, H-4′), 5.15 (s, 1H, NH), 6.17 (t, 1H, H-1′), 7.33 (s, 1H, H-6);^{13}C NMR (MeOH-d_4): δ 13.22 (CH_3), 28.53 (CH_2), 29.10 (CH_3), 36.53 (CH_2-$C_{carborane}$), 41.32 (CH_2N), 41.55 (C-2′), 62.72 (C-5′), 72.04 ($OC(CH_3)_3$), 74.09 (C-3′), 81.62 ($C_{carborane}$-C), 82.99 ($C_{carborane}$-C), 87.10 (C-1′), 88.84 (C-4′), 110.68 (C-5), 136.61 (C-6), 152.22 (C=O), 154.59 (C=O), 163.31 (C=O); MS (HR-ESI) $C_{20}H_{39}B_{10}N_3O_7Na$ $(M + Na)^+$ calcd: 564.3616, found: 564.3638.

3-{4-[(7-(*tert*-Butyloxy)carbonylamino)-*closo-m*-carboran-1-yl]butan-1-yl}thymidine (14). Compound **14** was prepared according to the procedure described for compound **12** using compound **8** (206 mg, 0.42 mmol), thymidine (252 mg, 1.04 mmol), and potassium carbonate (275 mg, 1.99 mmol). Purification by silica gel column chromatography (dichloromethane/acetone, 2:1) gave **14** as a white solid in 69% (160 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.35−1.38 (m, 2H, CH_2), 1.40 (s, 9H, $C(CH_3)_3$), 1.48−1.50 (m, 2H, CH_2), 1.90 (d, 3H, CH_3, J = 1.1 Hz), 1.92−1.97 (m, 2H, CH_2-$C_{carborane}$), 2.30−2.46 (m, 2H, H-2′), 3.80−3.94 (m, 4H, H-5′ and CH_2N), 3.98−4.00 (m, 1H,H H-3′), 4.59−4.61 (m, 1H, H-4′), 6.11 (t, 1H, H-1′, J = 6.8 Hz), 7.29 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR ($CDCl_3$): δ 13.27 (CH_3), 26.87 (CH_2), 27.09 (CH_2), 28.14 (CH_3), 36.47 (CH_2-$C_{carborane}$), 40.11 (CH_2N), 40.55 (C-2′), 62.45 (C-5′), 71.57 ($OC(CH_3)_3$), 71.84 (C-3′), 80.40 ($C_{carborane}$-C), 81.53 ($C_{carborane}$-C), 86.81 (C-1′), 88.00 (C-4′), 110.35 (C-5), 135.05 (C-6), 150.84 (C=O), 151.97 (C=O), 163.17 (C=O); MS (HR-ESI) $C_{21}H_{41}B_{10}N_3O_7Na$ $(M + Na)^+$ calcd: 578.3773, found: 578.3887.

3-{5-[(7-(*tert*-Butyloxy)carbonylamino)-*closo-m*-carboran-1-yl]pentan-1-yl}thymidine (15). Compound **15** was prepared according to the procedure described for compound **12** using compound **9** (380 mg, 0.76 mmol), thymidine (460 mg, 1.9 mmol), and potassium carbonate (420 mg, 3.04 mmol). Purification by silica gel column chromatography (dichloromethane/acetone, 2:1) gave **15** as a white solid in 79% (340 mg) yield. ^{1}H NMR (acetone-d_6): δ 1.23−1.32 (m, 2H, CH_2), 1.36 (s, 9H, $C(CH_3)_3$), 1.39−1.45 (m, 2H, CH_2), 1.52−1.61 (m, 2H, CH_2), 1.81−1.82 (d, 3H, CH_3, J = 1.2 Hz), 2.02−2.07 (m, 2H, CH_2-$C_{carborane}$), 2.20−2.25 (m, 2H, H-2′), 3.75−3.79 (m, 2H, H-5′), 3.81−3.87 (m, 2H, CH_2N), 3.90−3.94 (m, 1H, H-3′), 4.21−4.26 (t, 1H, OH-5′, J = 5.0 Hz), 4.38−4.40 (d, 1H, OH-3′, J = 4.2 Hz), 4.44−4.51 (m, 1H, H-4′), 6.33 (t, 1H, H-1′, J = 6.6 Hz), 7.49 (br s, 1H, NH), 7.81 (q, 1H, H-6, J = 1.2 Hz); ^{13}C NMR ($CDCl_3$): δ 13.03 (CH_3), 26.24 (CH_2), 26.94 (CH_2), 27.94 (CH_3), 31.24 (CH_2), 36.34 (CH_2-$C_{carborane}$), 40.21 (CH_2N), 40.85 (C-2′), 61.98 (C-5′), 70.97 ($OC(CH_3)_3$), 73.65 (C-3′), 80.56 ($C_{carborane}$-C), 81.11 ($C_{carborane}$-C), 86.37 (C-1′), 86.94 (C-4′), 109.81 (C-5), 134.55 (C-6), 150.70 (C=O), 151.98 (C=O), 163.27 (C=O); MS (HR-ESI) $C_{22}H_{43}B_{10}N_3O_7Na$ $(M + Na)^+$ calcd: 592.3999, found: 592.3986.

3-{6-[(7-(*tert*-Butyloxy)carbonylamino)-*closo-m*-carboran-1-yl]hexan-1-yl}thymidine (16). Compound **16** was prepared according to the procedure described for compound **12** using compound **10** (208 mg, 0.40 mmol), thymidine (262 mg, 1.08 mmol), and potassium carbonate (273 mg, 1.98 mmol). Purification by silica gel column chromatography (dichloromethane/acetone, 2:1) gave **16** as a white solid in 55% (128 mg) yield. ^{1}H NMR ($CDCl_3$): δ 1.18−1.29 (m, 6H, CH_2), 1.39 (s, 9H, $C(CH_3)_3$), 1.52−1.54 (m, 2H, CH_2), 1.86−1.89 (m, 2H, CH_2-$C_{carborane}$), 1.90 (d, 3H, CH_3, J = 1.0 Hz), 2.30−2.40 (m, 2H, H-2′), 3.79−3.92 (m, 4H, H-5′ and CH_2N), 3.97−4.00 (m, 1H, H-3′), 4.55−4.59 (m, 1H, H-4′), 6.15 (t, 1H, H-1′, J = 6.8 Hz), 7.32 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR ($CDCl_3$): δ 13.28 (CH_3), 26.37 (CH_2), 27.26 (CH_2), 28.13 (CH_3), 28.67 (CH_2), 29.71 (CH_2), 36.96 (CH_2-$C_{carborane}$), 40.08 (CH_2N), 41.15 (C-2′), 62.40 (C-5′), 71.52 ($OC(CH_3)_3$), 74.03 (C-3′), 80.45 ($C_{carborane}$-C), 81.59 ($C_{carborane}$-C), 86.86 (C-1′), 87.65 (C-4′), 110.33 (C-5), 134.86 (C-

6), 150.95 (C=O), 152.02 (C=O), 163.30 (C=O); MS (HR-ESI) $C_{23}H_{45}B_{10}N_3O_7Na$ $(M + Na)^+$ calcd: 606.4085, found: 606.4249.

3-{2-[(7-(*tert*-Butyloxy)carbonylamino)-*closo-m*-carboran-1-yl]ethyloxyethyl}thymidine (17). Compound **17** was prepared according to procedure described for compound **12** using compound **11** (160 mg, 0.32 mmol), thymidine (200 mg, 0.80 mmol), and potassium carbonate (175 mg, 1.28 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **17** as a white solid in 50% (90 mg) yield. ^{1}H NMR (acetone-d_6): δ 1.38 (s, 9H, $C(CH_3)_3$), 1.83 (d, 3H, CH_3, J = 1.2 Hz), 2.15−2.20 (m, 2H, CH_2-$C_{carborane}$), 2.22−2.26 (m, 2H, H-2′), 3.40 (t, 2H, OCH_2, J = 6.3 Hz), 3.57 (t, 2H, OCH_2, J = 6.0 Hz), 3.75−3.81 (m, 2H, H-5′), 3.94−3.96 (m, 1H, H-3′), 4.08 (t, 2H, CH_2N, J = 6.0 Hz), 4.26 (t, 1H, OH-5′, J = 5.1 Hz), 4.41 (d, 1H, OH-3′, J = 4.2 Hz), 4.47−4.50 (m, 1H, H-4′), 6.32−6.37 (t, 1H, H-1′, J = 6.9 Hz), 7.47 (br s, 1H, NH), 7.82−7.83 (d, 1H, H-6, J = 1.2 Hz); ^{13}C NMR (MeOH-d_4): δ 13.30 (CH_3), 28.27 (CH_3), 37.41 (CH_2-$C_{carborane}$), 40.40 (CH_2N), 41.15 (C-2′), 62.74 (C-5′), 67.45 (CH_2), 69.37 (CH_2), 71.80 ($OC(CH_3)_3$), 72.02 (C-3′), 79.45 ($C_{carborane}$-C), 80.79 ($C_{carborane}$-C), 86.40 (C-1′), 88.54 (C-4′), 109.79 (C-5), 135.62 (C-6), 151.67 (C=O), 153.26 (C=O) 163.75 (C=O); MS (HR-ESI) $C_{21}H_{41}B_{10}N_3O_8Na$ $(M + Na)^+$ calcd: 594.3788, found: 594.3777.

3-[2-(7-Amino-*closo-m*-carboran-1-yl)-ethan-1-yl]thymidine (18a). Trifluoroacetic acid (0.5 mL, 0.74 mmol) was added at to a solution of compound **12** (195 mg, 0.37 mmol) dissolved in dichloromethane (10 mL) 0 °C. The mixture was stirred at room temperature for 24 h. After evaporation, triethylamine (1 mL) and distilled water (10 mL) were added. (*Caution: It is necessary to remove CF_3COOH completely by evaporation to avoid the generation of toxic fumes due to acid−base reaction*). The residue was extracted with ethyl acetate (5 × 20 mL), and the combined organic layers were washed with brine and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (ethyl acetate/MeOH, 15:1) to give compound **18a** as a white solid in 85% (135 mg) yield. ^{1}H NMR (MeOH-d_4): δ 1.87 (d, 3H, CH_3, J = 1.2 Hz), 2.11−2.29 (m, 4H, CH_2-$C_{carborane}$ and H-2′), 3.72 (dd, 1H, H-5′, J = 12.0 and 3.1 Hz), 3.80 (dd, 1H, H-5′, J = 12.0 and 3.1 Hz), 3.86−3.93 (m, 3H, H-3′ and CH_2N), 4.34−4.38 (m, 1H, H-4′), 6.25 (t, 1H, H-1′, J = 6.5 Hz), 7.81 (d, 1H, H-6, J = 1.2 Hz); ^{13}C NMR (MeOH-d_4): δ 13.08 (CH_3), 34.03 (CH_2-$C_{carborane}$), 41.31 (CH_2N), 41.45 (C-2′), 62.71 (C-5′), 72.04 (C-3′), 74.68 ($C_{carborane}$-C), 87.13 (C-1′), 88.88 (C-4′), 89.93 ($C_{carborane}$-C), 110.61 (C-5), 136.66 (C-6), 151.90 (C-2), 164.95 (C-4); MS (HR-ESI) $C_{14}H_{29}B_{10}N_3O_5Na$ $(M + Na)^+$ calcd: 450.3016, found: 450.3022; RP-18: 19.01 min, RP-8: 18.47 min, >98% pure.

3-[3-(7-Amino-*closo-m*-carboran-1-yl)propan-1-yl]thymidine (19a). Compound **19a** was prepared according to the procedure described for compound **18a** using compound **13** (1.10 g, 2.03 mmol) and trifluoroacetic acid (2.74 mL, 4.06 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **19a** as a white solid in 87% (780 mg) yield. ^{1}H NMR (MeOH-d_4): δ 1.60−1.73 (m, 2H, CH_3), 1.90 (s, 3H, CH_3, J = 1.1 Hz), 1.95−2.02 (m, 2H, CH_2-$C_{carborane}$), 2.13−2.32 (m, 2H, H-2′), 3.69−3.84 (m, 4H, H-5′ and CH_2N), 3.88−3.93 (m, 1H, H-3′), 4.36−4.41 (m, 1H, H-4′), 6.29 (t, 1H, H-1′, J = 6.6 Hz), 7.82 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4): δ 13.16 (CH_3), 29.16 (CH_2), 35.30 (CH_2-$C_{carborane}$), 41.31 (CH_2N), 41.44 (C-2′), 62.74 (C-5′), 72.06 (C-3′), 74.71 ($C_{carborane}$-C), 87.12 (C-1′), 88.87 (C-4′), 91.18 ($C_{carborane}$-C), 110.68 (C-5), 136.62 (C-6), 152.24 (C-2), 165.31 (C-4); MS (HR-ESI) $C_{15}H_{31}B_{10}N_3O_5Na$ $(M + Na)^+$ calcd 464.3178, found 464.3184: RP-18:19.08 min, RP-8: 18.54 min, >98% pure.

3-[4-(7-Amino-*closo-m*-carboran-1-yl)butan-1-yl]thymidine (20a). Compound **20a** was prepared according to the procedure described for compound **18a** using compound **14** (95 mg, 0.16 mmol) and trifluoroacetic acid (0.22 mL, 0.32 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **20a** as a white solid in 69% (48 mg) yield. ^{1}H NMR (MeOH-d_4): δ 1.34−1.39 (m, 2H, CH_2), 1.49−1.55 (m, 2H, CH_2), 1.89−1.90 (d, 3H, CH_3, J = 1.1 Hz), 1.96−2.01 (m, 2H, CH_2-$C_{carborane}$), 2.15−2.29 (m, 2H, H-2′), 3.72 (dd, 1H, H-5′, J = 12.1 and 3.4 Hz), 3.79 (dd, 1H, H-5′, J = 12.1 and 3.4 Hz), 3.85−3.91 (m, 3H, CH_2N and H-3′), 4.37−4.39 (m, 1H, H-4′), 6.29 (t, 1H, H-1′, J = 6.4 Hz), 7.84 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4): δ 13.21 (CH_3), 27.99 (CH_2), 28.30 (CH_2), 37.46 (CH_2-$C_{carborane}$), 41.38 (CH_2N), 41.56 (C-2′), 62.76 (C-5′), 72.11 (C-3′), 75.36 ($C_{carborane}$-C), 87.16 (C-1′), 88.90 (C-4′), 89.45-($C_{carborane}$-C), 110.70 (C-5), 136.51 (C-6), 152.29 (C-2), 165.37 (C-4); MS (HR-ESI) $C_{16}H_{33}B_{10}N_3O_5Na$ $(M + Na)^+$ calcd: 478.3316, found: 478.3309; RP-18: 20.81 min, RP-8: 19.88 min, >98% pure.

3-[5-(7-Amino-*closo-m*-carboran-1-yl)pentan-1-yl]thymidine (21a). Compound **21a** was prepared according to the procedure described for compound **18a** using compound **15** (330 mg, 0.58 mmol) and trifluoroacetic acid (0.78 mL, 1.16 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **21a** as a white solid in 81% (220 mg) yield. ^{1}H NMR (MeOH-d_4): δ 1.22−1.30 (m, 2H, CH_2), 1.34−1.45 (m, 2H, CH_2), 1.51−1.60 (m, 2H, CH_2), 1.89 (d, 3H, CH_3, J = 1.2 Hz), 1.92−1.99 (m, 2H, CH_2-$C_{carborane}$), 2.19−2.25 (m, 2H, H-2′), 3.71 (dd, 1H, H-5′ J = 12.0 and 3.7 Hz), 3.79 (dd, 1H, H-5′ J = 12.0 and 3.7 Hz), 3.85−3.93 (m, 3H, H-3′ and CH_2N), 4.36−4.41 (m, 1H, H-4′), 6.29 (t, 1H, H-1′, J = 6.5 Hz), 7.82 (d, 1H, H-6, J = 1.2 Hz); ^{13}C NMR (acetone-d_6): δ 13.20 (CH_3), 27.37 (CH_2), 28.04 (CH_2), 30.73 (CH_2), 37.79 (CH_2-$C_{carborane}$), 41.31 (CH_2N), 42.01 (C-2′), 62.75 (C-5′), 72.09 (C-3′), 75.50 ($C_{carborane}$-C), 87.11 (C-1′), 88.86 (C-4′), 89.41($C_{carborane}$-C), 110.71 (C-5), 136.47 (C-6), 152.30 (C-2), 165.41 (C-4); MS (HR-ESI) $C_{17}H_{35}B_{10}N_3O_5Na$ $(M + Na)^+$ calcd: 492.3472, found: 492.3459; RP-18: 22.65 min, RP-8: 21.25 min, >98% pure.

3-[5-(7-Amino-*closo-m*-carboran-1-yl)hexan-1-yl]thymidine (22a). Compound **22a** was prepared according to the procedure described for compound **18a** using compound **16** (84 mg, 0.14 mmol) and trifluoroacetic acid (0.19 mL, 0.28 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **22a** as a white solid in 78% (53 mg) yield. ^{1}H NMR (MeOH-d_4): δ 1.21−1.28 (m, 4H, CH_2), 1.33−1.39 (m, 2H, CH_2), 1.53−1.58 (m, 2H, CH_2), 1.89 (d, 3H, CH_3, J = 1.1 Hz), 1.91−1.96 (m, 2H, CH_2-$C_{carborane}$), 2.15−2.29 (m, 2H, H-2′), 3.71 (dd, 1H, H-5′, J = 12.1 and 3.4 Hz), 3.79 (dd, 1H, H-5′, J = 12.1 and 3.4 Hz), 3.86−3.91 (m, 3H, CH_2N and H-3′), 4.37−4.39 (m, 1H, H-4′), 6.30 (t, 1H, H-1′, J = 6.9 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4): δ 13.20 (CH_3), 27.46 (CH_2), 28.32 (CH_2), 29.77 (CH_2), 30.67 (CH_2), 31.02 (CH_2), 37.90 (CH_2-$C_{carborane}$), 41.33 (CH_2N), 42.15 (C-2′), 62.76 (C-5′), 72.10 (C-3′), 75.58 ($C_{carborane}$-C), 87.11 (C-1′), 88.87 (C-4′), 89.40 ($C_{carborane}$-C), 110.71 (C-5), 136.46 (C-6), 152.31 (C-2), 165.41 (C-4); MS (HR-ESI) $C_{18}H_{37}B_{10}N_3O_5Na$ $(M + Na)^+$ calcd: 506.3629, found: 506.3811; RP-18: 24.76 min, RP-8: 23.35 min, >98% pure.

3-[2-(7-Amino-*closo-m*-carboran-1-yl)ethoxyethyl]thymidine (23a). Compound **23a** was prepared according to the procedure described for compound **18a** using compound **17** (60 mg, 0.10 mmol) and trifluoroacetic acid (0.14 mL, 0.20 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **23a** as a white solid in 85% (40 mg) yield. ^{1}H NMR (MeOH-d_4): δ 1.91 (d, 3H, CH_3, J = 1.2 Hz), 2.07−2.17 (m, 2H, CH_2-$C_{carborane}$), 2.21−2.29 (m, 2H, H-2′), 3.37 (m, 2H, OCH_2, J = 5.7 Hz), 3.59 (t, 2H, OCH_2, J = 5.6 Hz), 3.71 (dd, 1H, H-5′, J = 12.0 and 3.7 Hz), 3.79 (dd, 1H, H-5′, J = 12.0 and 3.7 Hz), 3.88−3.92 (m, 2H, H-3′), 4.14 (m, 2H, CH_2N, J = 5.7 Hz), 4.36−4.41 (m, 1H, H-4′), 6.32 (t, 1H, H-1′, J = 6.9 Hz), 7.85 (d, 1H, H-6, J = 1.2 Hz);^{13}C NMR (MeOH-d_4): δ 13.30 (CH_3), 37.73 (CH_2-$C_{carborane}$), 41.37 (CH_2N), 42.43 (C-2′), 62.78 (C-5′), 67.90 (CH_2), 70.08 (CH_2), 72.15 (C-3′), 73.26 ($C_{carborane}$-C), 87.16 (C-1′), 88.89 (C-4′), 89.74 ($C_{carborane}$-C), 110.77 (C-5), 136.70 (C-6), 152.44 (C-2), 165.53 (C-4); MS (HR-ESI) $C_{16}H_{33}B_{10}N_3O_6Na$ $(M + Na)^+$ calcd: 494.3264, found: 494.3250; RP-18: 18.08 min; RP-8: 17.61 min, >98% pure.

3-[2-(7-Ammonium-*nido-m*-carboran-1-yl)ethan-1-yl]-thymidine (18b). Tetrabutylbutylammonium fluoride hydrate (200 mg, 0.63 mmol) was added to a solution of compound **18a** (50 mg, 0.12 mmol) in tetrahydrofuran (2 mL) and stirred at 70 °C for 1 h. The progress of the reaction was monitored by

the IR. Distilled water (5 mL) was added at 0 °C, followed by the addition of hydrochloric acid (3.0 N) to adjust the pH to 2−3. The solution was extracted with ethyl acetate (5 × 20 mL), and the combined organic layers were washed with brine and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) to give compound **18b** in 70% (35 mg) yield. ^{1}H NMR (MeOH-d_4): δ −1.94 (br s, 1H, Hμ), 1.90 (d, 3H, CH_3, J = 1.1 Hz), 1.92−1.97 (m, 2H, CH_2-$C_{carborane}$), 2.16−2.32 (m, 2H, H-2′), 3.72 (m, 2H, H-5′), 3.88−3.93 (m, 1H, H-3′), 4.02−4.22 (m, 2H, CH_2N), 4.36−4.41 (m, 1H, H-4′), 6.27 and 6.36 (t, 1H, H-1′, J = 6.7 Hz), 7.81 and 7.82 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4): δ 13.28 (CH_3), 36.56 and 36.64 (*C*H_2-$C_{carborane}$), 41.21 and 41.42 (CH_2N), 44.70 and 44.82 (C-2′), 62.74 and 92.91 (C-5′), 72.00 and 72.12 (C-3′), 86.94 and 87.28 (C-1′), 88.84 (C-4′), 111.72 and 110.20 (C-5), 136.23 and 136.41 (C-6), 152.32 (C-2), 165.63 and 165.70 (C-4); ^{11}B NMR (MeOH-d_4): δ −33.41, −32.35, −20.19, −15.71, −2.24, −0.58; MS (HR-ESI) $C_{14}H_{30}B_9N_3O_5Na$ (M + Na)$^+$ calcd: 441.2968, found: 441.2964; RP-18: 14.21 and 14.43 min, RP-8: 14.49 and 14.65 min, >98% pure.

3-[3-(7-Ammonium-*nido*-*m*-carboran-1-yl)propan-1-yl]-thymidine (19b). Compound **19b** was prepared according to the procedure described for compound **18b** using compound **19a** (25 mg, 0.057 mmol) and tetrabutylbutylammonium fluoride hydrate (77 mg, 0.33 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) gave **19b** in 61% (15 mg) yield. ^{1}H NMR (MeOH-d_4); δ −1.86 (br s, 1H, Hμ) 1.54−1.82 (m, 4H, CH_2-$C_{carborane}$ and CH_2), 1.90 (d, 3H, CH_3, J = 1.1 Hz), 2.14−2.30 (m, 2H, H-2′), 3.72 (dd, 1H, H-5′, J = 12.0 and 3.1 Hz), 3.80 (dd, 1H,H-5′, J = 12.0 and 3.1 Hz), 3.89−3.94 (m, 3H, H-3′ and CH_2N), 4.36−4.42 (m, 1H, H-4′), 6.30 (t, 1H, H-1′, J = 6.8 Hz), 7.82 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4): δ 13.23 (CH_3), 31.74 (CH_2), 37.01 (*C*H_2-$C_{carborane}$), 41.35 (CH_2N), 42.81 (C-2′), 62.76 (C-5′), 72.07 (C-3′), 87.11 (C-1′), 88.85 (C-4′), 111.75 (C-5), 136.38 (C-6), 152.32 (C-2), 165.45 (C-4);^{11}B NMR (MeOH-d_4): δ −36.85, −35.15, −23.02, −18.93, −5.02, −3.16; MS (HR-ESI) $C_{15}H_{32}B_9N_3O_5Na$ (M + Na)$^+$ calcd: 455.3125, found: 455.3123; RP-18: 14.96 min, RP-8: 14.91 min, >98% pure.

3-[4-(7-Ammonium-*nido*-*m*-carboran-1-yl)butan-1-yl]-thymidine (20b). Compound **20b** was prepared according to the procedure described for compound **18b** using compound **20a** (45 mg, 0.099 mmol) and tetrabutylbutylammonium fluoride hydrate (105 mg, 0.33 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) gave **20b** in 66% (29 mg) yield. ^{1}H NMR (MeOH-d_4): δ −1.92 (br s, 1H, Hμ), 1.64−1.75 (m, 6H, CH_2-$C_{carborane}$ and CH_2), 1.90 (s, 3H, CH_3), 2.05−2.33 (m, 2H, H-2′), 3.72 (dd, 1H, H-5′, J = 12.0 and 3.7 Hz), 3.79 (dd, 1H, H-5′, J = 12.0 and 3.7 Hz), 3.88−3.91 (m, 3H, H-3′ and CH_2N), 4.36−4.41 (m, 1H, H-4′), 6.29 (t, 1H, H-1′, J = 6.6 Hz), 7.82 (s, 1H, H-6); ^{13}C NMR (MeOH-d_4): δ 13.24 (CH_3), 28.81 (CH_2), 31.18 (CH_2), 39.57 (*C*H_2-$C_{carborane}$), 41.38 (CH_2N), 42.58 (C-2′), 62.76 (C-5′), 72.08 (C-3′), 87.15 (C-1′), 88.87 (C-4′), 110.76 (C-5), 136.41 (C-6), 152.34 (C-2), 165.48 (C-4); ^{11}B NMR (D_2O): δ −33.94, −32.18, −20.29, −16.10, −1.88, −0.52; MS (HR-ESI) $C_{16}H_{34}B_9N_3O_5Na$ (M + Na)$^+$ calcd: 469.3282, found: 469.3274; RP-18: 15.38 min, RP-8: 15.43 min, >98% pure.

3-[5-(7-Ammonium-*nido*-*m*-carboran-1-yl)pentan-1-yl]thymidine (21b). Compound **21b** was prepared according to the procedure described for compound **18b** using compound **21a** (50 mg, 0.11 mmol) and tetrabutylbutylammonium fluoride hydrate (125 mg, 0.40 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) gave **21b** in 65% (33 mg) yield. ^{1}H NMR (MeOH-d_4): δ −1.88 (br s, 1H, Hμ), 1.32−1.38 (m, 2H, CH_2), 1.56−1.78 (m, 6H, CH_2-$C_{carborane}$ and CH_2), 1.90 (d, 3H, CH_3, J = 1.2 Hz), 2.13−2.33 (m, 2H, H-2′), 3.72 (dd, 1H, H-5′, J = 12.0 and 3.8 Hz), 3.79 (dd, 1H, H-5′, J = 12.0 and 3.8 Hz), 3.84−3.86 (m, 3H, CH_2N and H-3′), 4.36−4.41 (m, 1H, H-4′), 6.23 (t, 1H, H-1′, J = 6.9 Hz), 7.82 (d, 1H, H-6, J = 1.2 Hz); ^{13}C NMR (MeOH-d_4): δ 13.24 (CH_3), 28.28 (CH_2), 28.69 (CH_2), 33.69 (CH_2), 39.73 (*C*H_2-$C_{carborane}$), 41.34 (CH_2N), 42.55 (C-2′), 62.77 (C-5′), 72.09 (C-3′), 87.15 (C-1′), 88.87 (C-4′), 110.77 (C-5), 136.44 (C-6), 152.34 (C-2), 165.49 (C-4); MS (HR-ESI) $C_{17}H_{36}B_9N_3O_5Na$ (M + Na)$^+$ calcd: 483.3420, found: 483.3420, $C_{17}H_{35}B_9N_3O_5Na_2$ (M−H+Na_2)$^+$ calcd: 505.3240, found: 505.3240; RP-18: 16.36 min, RP-18: 16.12 min, >98% pure.

3-[6-(7-Ammonium-*nido*-*m*-carboran-1-yl)hexan-1-yl]-thymidine (22b). Compound **22b** was prepared according to the procedure described for compound **18b** using compound **22a** (40 mg, 0.083 mmol) and tetrabutylbutylammonium fluoride hydrate (100 mg, 0.32 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) gave **22b** in 53% (21 mg) yield. ^{1}H NMR (MeOH-d_4): δ −1.89 (br s, 1H, Hμ), 1.30−1.38 (m, 4H, CH_2), 1.64−1.79 (m, 6H, CH_2-$C_{carborane}$ and CH_2), 1.90 (d, 3H, CH_3, J = 1.1 Hz), 2.13−2.29 (m, 2H, H-2′), 3.71 (dd, 1H, H-5′, J = 12.0 and 3.0 Hz), 3.80 (dd, 1H, H-5′, J = 12.0 and 3.0 Hz), 3.90−3.92 (m, 3H, H-3′ and CH_2N), 4.36−4.41 (m, 1H, H-4′), 6.29 (t, 1H, H-1′, J = 6.5 Hz), 7.82 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4): δ 13.23 (CH_3), 28.06 (CH_2), 28.63 (CH_2), 30.69 (CH_2), 33.94 (CH_2), 39.78 (*C*H_2-$C_{carborane}$), 41.34 (CH_2N), 42.45 (C-2′), 62.77 (C-5′), 72.11 (C-3′), 87.12 (C-1′), 88.88 (C-4′), 110.74 (C-5), 136.43 (C-6), 152.33 (C-2), 165.46 (C-4); ^{11}B NMR (MeOH-d_4) δ −33.87, −32.15, −20.15, −15.94, −1.91, −0.32; MS (HR-ESI) $C_{18}H_{38}B_9N_3O_5Na$ (M + Na)$^+$ calcd: 497.3597, found: 497.3596; RP-18: 17.77 min, RP-8: 17.23 min, >98% pure.

3-[2-(7-Ammonium-*nido*-*m*-carboran-1-yl)ethoxyethyl]-thymidine (23b). Compound **23b** was prepared according to the procedure described for compound **18b** using compound **23a** (60 mg, 0.13 mmol) and tetrabutylbutylammonium fluoride hydrate (150 mg, 0.48 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 13:1, 1% acetic acid) gave **23b** in 50% (30 mg) yield. ^{1}H NMR (MeOH-d_4): δ −1.94 (br s, 1H, Hμ), 1.55−1.69 (m, 2H, CH_2-$C_{carborane}$), 1.90 (d, 3H, CH_3, J = 1.1 Hz), 2.15−2.33 (m, 2H, H-2′), 3.54−3.63 (m, 4H, OCH_2), 3.70 (dd, 1H, H-5′, J = 12.1 and 3.2 Hz), 3.79 (dd, 1H, H-5′, J = 12.1 and 3.2 Hz), 3.88−3.92 (m, 1H, H-3′), 4.13 (t, 2H, CH_2N, J = 6.2 Hz), 4.36−4.41 (m, 1H, H-4′), 6.29 (t, 1H, H-1′, J = 6.8 Hz), 7.81 (d, 1H, H-6, J = 1.1 Hz); ^{13}C NMR (MeOH-d_4): δ 13.25 (CH_3), 38.82 (*C*H_2-$C_{carborane}$), 41.38 (CH_2N), 41.40 (C-2′), 62.76 (C-5′), 67.77 (CH_2), 72.06 (C-3′), 74.57 (CH_2), 87.18 (C-1′), 88.86 (C-4′), 110.68 (C-5), 136.57 (C-6), 152.41 (C-2), 165.52 (C-4); ^{11}B NMR (D_2O): δ −33.86, −32.26, −20.23, −16.08, −2.00, −0.57; MS (HR-ESI) $C_{16}H_{34}B_9N_3O_6Na$ (M + Na)$^+$ calcd: 485.3232, found: 485.3232; RP-18: 14.48 min, RP-8: 14.63 min, >98% pure.

Phosphorylation Transfer Assay (PTA). PTAs with purified recombinant human TK1 and TK2 were carried out as described previously with minor modifications.[2,4] Briefly, thymidine and 3CTAs were dissolved in DMSO to produce stock solutions of various concentrations (30−140 mM). The reaction mixtures contained 10 μM of compounds for TK1 or 100 μM of compounds for TK2, 100 μM ATP (with 0.03 μM [γ-^{32}P]-ATP, Amersham Pharmacia Biotech, IL), 50 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 125 mM KCl, 10 mM DTT, and 0.5 mg/mL bovine serum albumin (BSA). The final concentrations of DMSO were set to 1%. The reaction mixtures were incubated at 37 °C for 20 min in the presence of 50 ng of enzyme. Subsequently, the enzyme was heat inactivated for 2 min at 95 °C. The reaction mixtures were centrifuged, and 1 μL sample portions were spotted on PEI-cellulose TLC plates (Merck). The TLC plates were placed overnight in a solvent system (isobutyric acid/ammonium hydroxide/water, 66:1:33). The radiolabeled spots were visualized by phospho-imager (Fuji Film, Science Lab., Image Gauge V3.3) and values for 3CTAs were expressed relative to that of Thd. The PTA is a procedure for the rapid screening of medium to large compound libraries providing phosphorylation rates for the assessment of the substrate characteristics of 3CTAs and other TK1 substrates. In our own studies, phosphorylation rates correlated appropriately with the catalytic efficiency (k_{cat}/K_M) of the phosphorylation of various TK1 substrates.[2,21] We have noticed that the phosphorylation rates of 3CTAs, determined in different PTAs, can vary in the range of ≤25% when

different recombinant TK1 preparation were used.[2,5,48,49] We therefore screened compound libraries in a single PTA using the same recombinant enzyme batch.

Tumor Models and in Vivo Experiments.[20] BPA (98% pure) was purchased from Rysor Science Inc (Raleigh, NC). **N5-2OH** (>97% pure) was synthesized according to a previously reported procedure (see Supporting Information for HPLC purity verification of **N5-2OH**).[2] The L929 (American Type Culture Collection #CCL1) cell line was one of the first to be established in continuous culture and clone 929 was the first cloned strain that was developed. The parent L strain was derived from normal subcutaneous areolar and adipose tissue from a C3H/An mouse, and clone 929, which is TK1 (+) was from the 95th subculture generation of the parent strain. Its TK1 (−) counterpart (ATCC #CCL1.3 L-M) was derived from the wild-type cell line L929. Both can be propagated in vitro as continuous cell lines, the latter in the presence of 5-bromo-2′-deoxyuridine (BrdUrd). Recently, the in vitro TK1 activities of L929 (wt) and L929 TK1 (−) cells have been determined to be 228 and 9 pmol/mg/min, respectively.[20] When implanted into nude mice, tumors resulting from L929 TK1 (−) cells maintained a TK1 (−) phenotype at least over a period of 2 weeks.[20] L929 (wt) and TK1 (−) tumors were established in nude mice by implanting 10^6 cells subcutaneously (sc). Two weeks later, the tumors had attained a weight of 0.3 to 1.1 g. Mice were injected intratumoraly (it) over a period of 2 min with quantities **N5-2OH**, **19b**, and BPA equivalent to 50 μg of boron solubilized in 15 μL of 70% aqueous DMSO, 10 μL of 24% aqueous DMSO, and 15 μL of PBS, respectively. This was followed by a second injection 2 h later. In both injections, 0.17 mol equival of 5-fluoro-2′-deoxyuridine (FdUrd) was co-injected to suppress the de novo synthesis of thymidine nucleotides.[50] Boron concentrations in tissues were determined by direct current plasma atomic emission spectroscopy (DCP-AES).[51] Each point represents the arithmetic mean ± SD of four mice.

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Supporting Information Available: Reversed phase C8 (RP-8) and C18 (RP-18) chromatograms of target compounds (**1**, **4**, **18a**−**23a**, and **18b**−**23b**) and **N5-2OH** for purity verification are available free of charge via the Internet at http://pubs.acs.org.

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