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Synthesis, Biological Evaluation, and Radioiodination of Halogenated *closo*-Carboranylthymidine Analogues[1]

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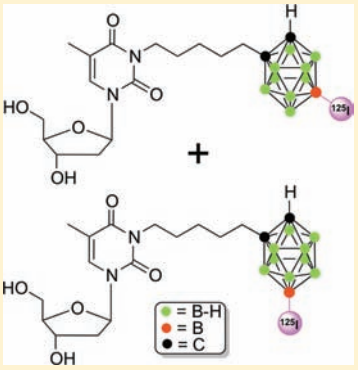
Supporting Information

ABSTRACT: The synthesis and initial biological evaluation of 3-carboranylthymidine analogues (3CTAs) that are (radio)halogenated at the *closo*-carborane cluster are described. Radiohalogenated 3CTAs have the potential to be used in the radiotherapy and imaging of cancer because they may be selectively entrapped in tumor cells through monophosphorylation by human thymidine kinase 1 (hTK1). Two strategies for the synthesis of a ^{127}I-labeled form of a specific 3CTA, previously designated as **N5**, are described: (1) direct iodination of **N5** with iodine monochloride and aluminum chloride to obtain **N5**-^{127}**I** and (2) initial monoiodination of *o*-carborane to 9-iodo-*o*-carborane followed by its functionalization to **N5**-^{127}**I**. The former strategy produced **N5**-^{127}**I** in low yields along with di-, tri-, and tetraiodinated **N5** as well as decomposition products, whereas the latter method produced only **N5**-^{127}**I** in high yields. **N5**-^{127}**I** was subjected to nucleophilic halogen- and isotope-exchange reactions using Na$^{79/81}$Br and Na^{125}I, respectively, in the presence of Herrmann's catalyst to obtain **N5**-$^{79/81}$**Br** and **N5**-^{125}**I**, respectively. Two intermediate products formed using the second strategy, 1-(*tert*-butyldimethylsilyl)-9-iodo-*o*-carborane and 1-(*tert*-butyldimethylsilyl)-12-iodo-*o*-carborane, were subjected to X-ray diffraction studies to confirm that substitution at a single carbon atom of 9-iodo-*o*-carborane resulted in the formation of two structural isomers. To the best of our knowledge, this is the first report of halogen- and isotope-exchange reactions of B-halocarboranes that have been conjugated to a complex biomolecule. Human TK1 phosphorylation rates of **N5**, **N5**-^{127}**I**, and **N5**-$^{79/81}$**Br** ranged from 38.0% to 29.6% relative to that of thymidine, the endogenous hTK1 substrate. The in vitro uptake of **N5**, **N5**-^{127}**I**, and **N5**-$^{79/81}$**Br** in L929 TK1(+) cells was 2.0, 1.8, and 1.4 times greater than that in L929 TK1(−) cells.



INTRODUCTION

Radiopharmaceuticals containing carbon-bound radiohalogens play an important role in a variety of therapy and imaging applications.[2] There is, however, a convincing body of evidence indicating that most radiopharmaceutical biomolecules (e.g., antibodies, carbohydrates, growth factors) that contain radiolabeled boron clusters are less susceptible to dehalogenation under physiological conditions than their counterparts containing conventional radiohalogen−carbon bonds.[3] The vast majority of these radiopharmaceuticals possess negatively charged boron clusters, such as *nido*-carborane(1−) ($C_2B_9H_{12}^-$), *closo*-monocarborane(1−) ($CB_{11}H_{12}^-$), decaborate(2) ($B_{10}H_{10}^{2-}$), or dodecaborate ($B_{12}H_{12}^{2-}$).[3] By analogy to electrophilic radiohalogenation of tyrosine residues in proteins,[2] labeling of these negatively charged boron clusters mainly was carried out with electrophilic halogen species produced in situ by the action of Chloramine T or Iodogen.[3] Nucleophilic labeling is another widely used strategy for the introduction of carbon-bound radiohalogens in biomolecules.[2,4,5] Methods employing iron-, copper-, and palladium-catalyzed nucleophilic halogen

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exchange at simple boron cluster structures were developed,[6−11] but they have not as yet been applied to the radiohalogenation of more complex biologically active carborane-containing compounds. Nucleophilic halogen exchange can be carried out at hydrophobic neutral boron clusters and thus could be a useful alternative in the radiohalogenation of low-molecular-weight boron-cluster-containing bioconjugates that may rely on hydrophobic properties for biological activity.[12]

Thymidine (dThd) analogues with a hydrophobic boron cluster (*closo*-carborane) attached through spacers to the N3 position (3-carboranylthymidine analogues or 3CTAs) may be good examples for such compounds. Both boronated and nonboronated N3-substituted dThd analogues have attracted considerable attention in a variety of biomedical applications in recent years.[13−21] 3CTAs have been evaluated as boron delivery agents for boron neutron capture therapy (BNCT) of cancer.[19,22] One of the lead 3CTAs that was identified is **N5** (**3**; Figure 1 and Scheme 1).[14,23] This agent appears to selectively accumulate within cancer cells following monophosphorylation by human thymidine kinase 1 (hTK1), which is overexpressed in cancer cells. This form of selective entrapment of nucleoside analogues in tumor cells has been referred to as "kinase mediated trapping" (KMT).[13] Furthermore, 3CTAs did not appear to be substrates of nucleoside-catabolizing enzymes such as 5′-nucleotidases (5′-NTs) and nucleoside phosphorylases, and preliminary data indicated that these compounds might pass cell membranes by passive diffusion.[12,14,23] It is conceivable that cage-monoradioiodinated forms of **3** (e.g., **N5-^{125}I** [**13a** or **13b**, Scheme 3]) could be used in the radiotherapy and imaging of cancer, as well as for BNCT-related biodistribution/pharmacokinetic experiments. They may be superior to conventional radioiodinated nucleosides, such 5-[^{125}I]iodo-2′-deoxyuridine.[24−27] Under physiological conditions, the latter is cleaved within a few minutes by nucleoside phosphorylases to 5-[^{125}I]iodoracil. This is followed by rapid dehalogenation, which can lead to the accumulation of significant quantities of radioiodine in the thyroid and the stomach.[24−27]

In this paper, we describe strategies for the controlled introduction of a single ^{127}I atom to the *closo*-*o*-carborane cluster of **3**, the halogen and isotope exchange of iodine-127 to bromine-79/81 and iodine-125, respectively, via palladium-catalyzed nucleophilic halogen/isotope exchange, and the initial biological evaluation of cage-halogenated forms of **3** in phosphoryl transfer assays (PTAs) with hTK1 and in cell uptake experiments with L929 TK1(+) and L929 TK1(−) cells. To the best of our knowledge, this is the first description of this type of nucleophilic halogen/isotope-exchange reaction at a neutral boron cluster in a complex biomolecule. The methodology that we have developed could be applicable for the synthesis of a wide range of low-molecular-weight bioconjugates containing neutral radioiodinated boron clusters for bioimaging and the treatment of cancer.

EXPERIMENTAL SECTION

NMR spectra were obtained on a Bruker Avance 400 at The Ohio State University College of Pharmacy (400 MHz for ^{1}H, 100 MHz for ^{13}C, and 128 MHz for ^{11}B). Chemical shifts (δ) are reported in parts per million from internal deuterated chloroform, deuterated acetone, or an external $BF_3{\cdot}Et_2O$ standard. Coupling constants are reported in hertz. High-resolution electron spray ionization (HR-ESI) mass spectrometry (MS) spectra were obtained at The Ohio State University Campus Chemical Instrumentation Center on a Micromass Q-TOF II and a Micromass LCT spectrometer and at the University of Illinois Mass Spectrometry Laboratory (Urbana−Champaign, Illinois) on a Waters Q-TOF Ultima tandem quadrupole/time-of-flight spectrometer. Electron impact (EI) MS spectra were obtained at the University of Illinois Mass Spectrometry Laboratory (Urbana−Champaign, Illinois) on a Micromass 70-VSE double-focusing-sector spectrometer on TSSPro3.0 system. For all carborane-containing compounds, the mass of the most intensive peak of the isotopic pattern was reported for a 80% ^{11}B to 20% ^{10}B distribution. Measured patterns agreed with calculated patterns. Melting points were measured in sealed capillaries on a Thomas−Hoover capillary melting point apparatus and are uncorrected. Silica gel 60 (0.063−0.200 mm) from Merck was used for gravity column chromatography, whereas silica gel 60 (0.015−0.049 mm) from EM Science was used for flash column chromatography. Precoated glass-backed thin-layer chromatography (TLC) plates with silica gel 60 F254 (0.25-mm layer thickness) from Dynamic Adsorbents (Norcross, GA) were used for TLC studies. For all nonradioactive materials, (semi)preparative high-performance liquid chromatography (HPLC) purification was performed either with a Gemini 5μ C18 column (21.20 mm ×250 mm, 5 μm particle size), supplied by Phenomenex Inc. (Torrance, CA), or with a Supelco Discovery HS C18 column (10 mm ×250 mm, 10 μm particle size), supplied by Sigma-Aldrich (St. Louis, MO). A LiChrocart 250-4 HPLC cartridge packed with a LiChrospher RP-18 stationary phase (4 mm ×250 mm, 5 μm particle size), supplied by EM Science (Gibbstown, NJ), was used for analytical reversed-phase chromatography. For all radioactive materials, semipreparative HPLC purification was performed with a Supelco Discovery HS C18 column (10 mm × 250 mm, 10 μm particle size). A Beckman Ultrasphere column (4.6 mm × 250 mm, 5 μm particle size), supplied by Beckmann Coulter Inc. (Brea, CA), was used for analytical reversed-phase chromatography. Further general experimental conditions are provided in the Supporting Information.

5-(*o*-Carboran-1-yl)pentyl 4-Methylbenzenesulfonate (2).[28] 6-Heptynyl 4-methylbenzenesulfonate (**1**;[29] 1.0 g, 3.7 mmol), decaborane (240 mg, 2 mmol), and 1-butyl-3-methylimidazolium chloride (bmim$^+$Cl$^-$; 300 mg, 1.72 mmol) were vigorously stirred in anhydrous toluene at 120 °C for 10 min. Subsequently, the reaction mixture was diluted with CH_2Cl_2 and filtered through silica gel. The filtrate was evaporated, and the residue was purified by column chromatography using hexanes/EtOAc (2:1) to afford **2** (420 mg, 55%) as a white solid. ^{1}H NMR ($CDCl_3$) data were the same as those obtained previously for the same compound using a different procedure.[28] The measured melting point was slightly lower than the one previously reported (105−106 °C vs 109−110 °C).

3-[5-(*o*-Carboran-1-yl)pentyl]thymidine (3).[28] To a solution of **2** (650 mg, 1.7 mmol) in *N*,*N*-dimethylformamide (DMF)/acetone (1:1, 15 mL) were added dThd (500 mg, 2.06 mmol) and potassium carbonate (700 mg, 5.07 mmol). The solution was stirred for 24 h at 50 °C. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography using CH_2Cl_2/CH_3COCH_3 (4:1) as the solvent system to give compound **3** (490 mg, 64%) as a waxlike solid. Spectroscopic data were consistent with those previously reported for the same compound.[28]

3-[5-(9-Iodo-*o*-carboran-1-yl)pentyl]thymidine and 3-[5-(12-Iodo-*o*-carboran-1-yl)pentyl]thymidine (4a/4b, [N5-I]) [Strategy 1]. To a stirred solution of **3**[28] (25 mg, 0.055 mmol) and $AlCl_3$ (73 mg, 0.55 mmol) in anhydrous CH_2Cl_2 was added dropwise ICl (1 M in CH_2Cl_2; 55 μL, 0.055 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, quenched with a saturated solution of $Na_2S_2O_3$, and extracted with CH_2Cl_2. The organic layer was separated and the aqueous layer washed two times with 10 mL of CH_2Cl_2. The combined organic layers were washed with brine, dried over magnesium sulfate, and evaporated in vacuo. The product was crudely separated from the decomposition products by column chromatography using CH_2Cl_2/MeOH (10:1) or, alternatively, PhMe/CH_3CN (3:2) as the solvent system. R_f = 0.34 (CH_2Cl_2/MeOH, 10:1). Final purification, primarily by separation from the diiodinated product, was accomplished by preparative RP-18 HPLC (acetonitrile/water, 60:40) to furnish **4a**/**4b** (5.0 mg, 16%) as a white foam. ^{1}H NMR (CD_3COCD_3): δ 0.6−4.0 (m, 9H, BH), 1.32 (m, 4H, $-CH_2$), 1.57 (m, 8H, CH_2CH_2), 1.83 (s, 6H, $-CH_3$), 2.25−2.39 (m, 8H, $-CH_2C_{carborane}$ and H-2′), 3.78 (d, 4H, H-5′, J = 2.7 Hz), 3.86

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(m, 4H, $-CH_2N$), 3.93 (m, 2H, H-3′), 4.26 (m, 2H, −OH), 4.41 (d, 2H, H-4′, J = 3.2 Hz), 4.49 (s, 2H, −OH), 4.90 (s, 1H, $HC_{carborane}$), 5.10 (s, 1H, $HC_{carborane}$), 6.34 (t, 2H, H-1′, J = 6.8 Hz), 7.83 (s, 2H, H-6). $^{11}B\{^1H\}$ NMR (CD_3COCD_3): δ −17.79 (s, 1B, B12−I, B9−I), −16.15 (s, 1B, B9−I/B12−I), −11.04 (m, 12B, B3, B4, B5, B6, B7, B11), −7.19 (s, 4B, B8, B10), −3.96 (s, 1B, B12−H/B9−H), −0.58 (s, 1B, B9−H/B12−H). ^{11}B NMR (CD_3COCD_3): δ −17.93 (s, 1B), −16.26 (s, 1B), −11.17 (m, 12B), −7.33 (d, 4B, J = 156.2 Hz), −4.10 (d, 1B, J = 145.7 Hz), −0.70 (d, 1B, J = 153.5 Hz). HPLC retention time = 9.91 min (RP-18 analytical HPLC, 1 mL flow rate, solvent system CH_3CN/H_2O, 60:40, isocratic elution). MS (HR-ESI) for $C_{17}H_{33}B_{10}IN_2O_5$ [$(M + Na)^+$]. Calcd: m/z 603.2324. Found: m/z 603.2344. **N5-I$_2$**: HPLC retention time = 11.21 min (RP-18 analytical HPLC, 1 mL flow rate, solvent system CH_3CN/H_2O, 60:40, isocratic elution). MS (HR-ESI) for $C_{17}H_{32}B_{10}I_2N_2O_5$ [$(M + Na)^+$]. Calcd: m/z 729.1259. Found: m/z 729.1310.

3-[5-(8,9,10,12-Tetraiodo-*o*-carboran-1-yl)pentyl]thymidine (5, N5-I$_4$). To a stirred solution of **3**[28] (100 mg, 0.22 mmol) and $AlCl_3$ (440 mg, 3.3 mmol) in anhydrous CH_2Cl_2 was added dropwise a 1 M ICl solution in CH_2Cl_2 (1.1 mL, 1.1 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and then refluxed for 2 days. The reaction mixture was quenched with a saturated solution of $Na_2S_2O_3$ and extracted with CH_2Cl_2. The organic layer was separated, and the aqueous layer was washed with 2 × 10 mL of CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The product was crudely separated from the decomposition products by column chromatography using CH_2Cl_2/MeOH (10:1) or, alternatively, PhMe/CH_3CN (3:2) as the solvent system. R_f = 0.34 (CH_2Cl_2/MeOH, 10:1). Final purification, primarily by separation from the triiodinated product, was accomplished by preparative RP-18 HPLC (CH_3CN/H_2O, 60:40, isocratic elution) to furnish **5** (27 mg, 13%) as a yellow solid. HPLC retention time = 13.81 min (RP-18 analytical HPLC, 1 mL flow rate, solvent system CH_3CN/H_2O, 60:40, isocratic elution). ^1H NMR (CD_3COCD_3): δ 0.6−4.0 (m, 6H, BH), 1.33 (m, 2H, $-CH_2$), 1.61 (m, 4H, $-CH_2CH_2$), 1.83 (s, 3H, $-CH_3$), 2.23 (dd, 2H, H-2′, J = 4.7 and 6.7 Hz), 2.45 (m, 2H, $-CH_2C_{carborane}$), 3.78 (m, 2H, H-5′), 3.88 (m, 2H, $-CH_2N$), 3.94 (dd, 1H, H-3′, J = 3.1 and 6.1 Hz), 4.27 (s, 1H,−OH), 4.42 (s, 1H, −OH), 4.49 (s, 1H, H-4′), 5.72 (s, 1H, $HC_{carborane}$), 6.34 (t, 1H, H-1′, J = 6.8 Hz), 7.84 (s, 1H, H-6). $^{11}B\{^1H\}$ NMR (CD_3COCD_3): δ −17.8 (s, 4B, B8−I, B9−I, B10−I, and B12−I), −12.1 (s, 1B), −10.2, −8.4 (m, 5B). ^{11}B NMR (CD_3COCD_3): δ −17.9 (s, 4B, B8, B9, B10, and B12), −12.1 (d, 1B, J = 205.2 Hz), −10.1, −8.3 (m, 5B). MS (HR-ESI). $C_{17}H_{30}B_{10}I_4N_2O_5$ [$(M + H)^+$]. Calcd: m/z 960.9371. Found: m/z 960.9373. **N5-I$_3$**: HPLC retention time = 13.60 min (RP-18 analytical HPLC, 1 mL flow rate, solvent system CH_3CN/H_2O, 60:40, isocratic elution). MS (HR-ESI) for $C_{17}H_{31}B_{10}I_3N_2O_5$ [$(M + Na)^+$]. Calcd: m/z 855.0226. Found: m/z 855.0277.

9-Iodo-*o*-carborane (7).[30,31] To a solution of *o*-carborane (**6**; 2.0 g, 14 mmol) in anhydrous CH_2Cl_2 was added dropwise ICl (27.7 mmol, 27.7 mL of a 1 M solution in CH_2Cl_2) at 25 °C. The reaction mixture was stirred for 5 h at 40 °C, quenched with a saturated aqueous solution of $Na_2S_2O_3$, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by column chromatography to afford **7** (2.7 g, 72%) as a off-white solid. R_f = 0.38 (hexanes/ethyl acetate, 10:4). ^1H NMR (CD_3COCD_3): δ 1.53−3.21 (m, 9H, BH), 4.74 (s, 1H, $HC_{carborane}$), 4.95 (s, 1H, $HC_{carborane}$). $^{11}B\{^1H\}$ NMR (CD_3COCD_3): δ −16.5 (s, 1B, B9−I), −13.7 (s, 2B, B3, B6), −12.8 (s, 2B, B4, B5), −12.1 (s, 2B, B7, B11), −7.3 (s, 2B, B8, B10), −1.1 (s, 1B, B12). ^{11}B NMR (CD_3COCD_3): δ −16.5 (s, 1B), −13.1 (m, 6B), −7.3 (d, 2B, J = 155.0 Hz), −1.2 (d, 1B, J = 151.2 Hz). MS (HR-EI) for $C_2B_{10}H_9I$ (M^+). Calcd: m/z 270.0909. Found: m/z 270.0912.

1-(*tert*-Butyldimethylsilyl)-9-iodo-*o*-carborane (8a) and 1-(*tert*-Butyldimethylsilyl)-12-iodo-*o*-carborane (8b). To a stirred solution of **7** (2.1 g, 7.8 mmol) in anhydrous tetrahydrofuran (THF) at −78 °C was added dropwise a 2.5 M solution of *n*-BuLi in hexanes (3.26 mL, 8.15 mmol). The reaction mixture was allowed to stir for 30 min at room temperature and cooled to 0 °C, and *tert*-butyldimethylsilyl chloride (TBDMSCl; 1.29 g, 8.55 mmol) in 15 mL of anhydrous THF was added dropwise. The solution was refluxed overnight, carefully quenched with 10 mL of water, and extracted with 60 mL of diethyl ether. The layers were separated, and the aqueous layer was extracted with 2 × 30 mL of diethyl ether. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude residue was purified by column chromatography using pentane to afford **8a**/**8b** as colorless oil (2.0 g, 66%). R_f = 0.24−0.31. ^1H NMR (CD_3COCD_3): δ 0.30 (s, 6H, $-Si(CH_3)_2$), 0.33 (s, 6H, $-Si(CH_3)_2$), 1.04 (s, 9H, $-C(CH_3)_3$), 1.07 (s, 9H, $-C(CH_3)_3$), 1.47−2.23 (m, 9H, BH), 4.52 (s, 1H, $HC_{carborane}$), 4.74 (s, 1H, $HC_{carborane}$). MS (HR-EI) for $C_8H_{25}B_{10}ISi$ (M^+). Calcd: m/z 384.1774. Found: m/z 384.1774.

A quantity of 100 mg of the mixture of **8a** and **8b** was separated by column chromatography using pentane as the solvent system to give 58 mg of **8a** (R_f = 0.31; mp 105−106 °C) and 31 mg of **8b** (R_f = 0.24; mp 125−126 °C). **8a**. ^1H NMR (CD_3COCD_3): δ 0.33 (s, 6H, $-Si(CH_3)_2$), 1.09 (s, 9H, $-C(CH_3)_3$), 1.47−2.23 (m, 9H, BH), 4.77 (s, 1H, $HC_{carborane}$). $^{11}B\{^1H\}$ NMR (CD_3COCD_3): δ −15.0 (s, 1B, B9−I), −11.7 (m, 4B, B3, B4, B5, B6), −8.9 (s, 2B, B7, B11), −4.6 (s, 2B, B8, B10), 2.3 (s, 1B, B12). ^{11}B NMR (CD_3COCD_3): δ −15.0 (s, 1B), −10.4 (m, 6B), −4.6 (d, 2B, J = 152.6 Hz), 2.3 (d, 1B, J = 157.9 Hz, B12). MS (HR-EI) for $C_8H_{25}B_{10}ISi$ (M^+). Calcd: m/z 384.1774. Found: m/z 384.1758. **8b**. ^1H NMR (CD_3COCD_3): δ 0.31 (s, 6H, $-Si(CH_3)_2$), 1.04 (s, 9H, $-C(CH_3)_3$), 1.47−2.23 (m, 9H, BH), 4.55 (s, 1H, $HC_{carborane}$). $^{11}B\{^1H\}$ NMR (CD_3COCD_3): δ −14.4 (s, 1B, B12−I), −12.3 (m, 4B, B3, B4, B5, B6), −11.0 (m, 2B, B7, B11), −5.2 (s, 2B, B7, B11), −0.2 (s, 1B, B9). ^{11}B NMR (CD_3COCD_3): δ −14.3 (s, 1B), −13.0 (s, 1B), −10.4 (m, 1B, J = 76.7 and 168.5 Hz), −5.2 (d, 1B, J = 154.3 Hz), −0.3 (d, 1B, J = 152.1 Hz). MS (HR-EI) for $C_8H_{25}B_{10}ISi$ (M^+). Calcd: m/z 384.1774. Found: m/z 384.1791.

1-(*tert*-Butyldimethylsilyl)-2-[5-(*tert*-butyldimethylsilyloxy)-pentyl]-9-iodo-*o*-carborane and 1-(*tert*-Butyldimethylsilyl)-2-[5-(*tert*-butyldimethylsilyloxy)pentyl]-12-iodo-*o*-carborane (9a/9b). To a stirred solution of a mixture of **8a** and **8b** (6.52 g, 17 mmol) in 35 mL of anhydrous THF at −78 °C was added dropwise a 2.5 M solution of *n*-BuLi in hexanes (8.48 mL, 21.2 mmol). The reaction mixture was stirred for 30 min at room temperature and then cooled to 0 °C, and TBDMSCl (8.21 g, 22 mmol) dissolved in THF (20 mL) was added dropwise. The solution was stirred for 24 h, quenched with 20 mL of water, and extracted with 60 mL of diethyl ether. The separated aqueous layer was extracted with 2 × 30 mL of diethyl ether, and the combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude residue was purified by flash chromatography to afford **9a**/**9b** (6.5 g, 66%). R_f = 0.72 (pentane/diethyl ether, 30:1). ^1H NMR (CD_3COCD_3): δ 0.05 (s, 6H, $-Si(CH_3)_2$), 0.05 (s, 6H, $-Si(CH_3)_2$), 0.39 (s, 6H, $-Si(CH_3)_2$), 0.43 (s, 6H, $-Si(CH_3)_2$), 0.89 (s, 9H, $-C(CH_3)_3$), 0.90 (s, 9H, $-C(CH_3)_3$), 1.09 (s, 9H, $-C(CH_3)_3$), 1.12 (s, 9H, $-C(CH_3)_3$), 1.34−1.67 (m, 12H, $CH_2CH_2CH_2$), 1.3−3.27 (m, 18H, BH), 2.22 (m, 2H, $CH_2C_{carborane}$), 2.34 (m, 2H, $CH_2C_{carborane}$), 3.63 (dd, 4H, $-CH_2OTBDMS$, J = 6.2 and 13.2 Hz). $^{11}B\{^1H\}$ NMR (CD_3COCD_3): δ −15.9 (s, 1B, B12−I/B9−I), −14.2 (s, 1B, B9−I/B12−I), −9.0 (m, 12B, B3, B4, B5, B6, B7, B11), −5.3 (m, 4B, B8, B10), −2.5 (s, 1B, B12−H/B9−H), 2.0 (s, 1B, B9−H/B12−H). ^{11}B NMR (CD_3COCD_3): δ −15.9 (s, 1B), −14.2 (s, 1B), −9.2 (m, 12B), −5.3 (m, 4B), −2.4 (d, 1B, J = 148.6 Hz), 2.1 (d, 1B, J = 148.8 Hz). MS (HR-EI) for $C_{19}H_{49}B_{10}IOSi_2$ [$(M - 56)^-$]. Calcd: m/z 528.2744. Found: m/z 528.2760.

5-(9-Iodo-*o*-carboran-1-yl)pentan-1-ol and 5-(12-Iodo-*o*-carboran-1-yl)pentan-1-ol (10a/10b). To a solution of **9a**/**9b** (2.37 g, 4.05 mmol) in 40 mL of THF was added dropwise at −78 °C a 1.0 M solution of tetrabutylammonium fluoride (TBAF) in THF (6.1 mL, 6.1 mmol). The reaction mixture was stirred at 4 °C for 30 min, acidified with 10% methanolic/HCl (20 mL), and then stirred for an additional 30 min at 4 °C. The reaction mixture was extracted with ethyl acetate (3 × 35 mL), and the combined organic layers were washed with water, then saturated aqueous $NaHCO_3$, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (hexanes/ethyl acetate, 3:7) to furnish **10a**/**10b** (1.1 g, 76%). R_f = 0.54 (hexanes/ethyl acetate, 3:7). ^1H NMR (CD_3COCD_3): δ 1.28−1.59 (m, 12H, $-CH_2CH_2CH_2$), 1.3−3.27 (m, 18H, BH), 2.25 (m, 2H, $-CH_2C_{carborane}$), 2.38 (m, 2H, $-CH_2C_{carborane}$), 3.50 (dd, 4H, $-CH_2OH$, J = 5.3 and 10.2 Hz), 4.88 (br, 1H, $HC_{carborane}$), 5.09 (br, 1H, $HC_{carborane}$). $^{11}B\{^1H\}$ NMR (CD_3COCD_3):

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δ −18.4 (s, 1B, B12−I/B9−I), −16.7 (s, 1B, B9−I/B12−I), −11.7 (m, 12B, B3, B4, B5, B6, B7, B11), −7.7 (m, 4B, B8, B10), −4.4 (s, 1B, B12−H/B9−H), −1.15 (s, 1B, B9−H/B12−H). ^{11}B NMR (CD_3COCD_3): δ −18.1 (s, 1B), −16.4 (s, 1B), −11.3 (m, 14B), −7.3 (m, 2B, J = 152.5 Hz), −4.1 (d, 1B, J = 153.3 Hz), −0.7 (d, 1B, J = 150.4 Hz). MS (HR-EI) for $C_7H_{21}B_{10}IO$ (M^+). Calcd: m/z 356.1641. Found: m/z 356.1647.

5-(9-Iodo-*o*-carboran-1-yl)pentyl 4-Methylbenzenesulfonate and 5-(12-Iodo-*o*-carboran-1-yl)pentyl 4-Methylbenzenesulfonate (11a/11b). To a solution of **10a**/**10b** (1.65 g, 4.63 mmol) in CH_2Cl_2 (12 mL) was added triethylamine (0.84 mL, 6 mmol) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP; 113 mg, 0.93 mmol). A solution of *p*-toluenesulfonyl chloride (1.33 g, 6.95 mmol) in 13 mL of CH_2Cl_2 was added at 0 °C, and the reaction mixture was stirred at room temperature for 7 h. The reaction was quenched with a saturated NH_4Cl aqueous solution; the organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography to afford **11a**/**11b** (2.2 g, 93%) as an oil. R_f = 0.65 (hexanes/ethyl acetate, 1:1). ^{1}H NMR (CD_3COCD_3): δ 1.27−1.69 (m, 12H, $-CH_2CH_2CH_2$), 1.3−3.27 (m, 18H, BH), 2.23 (m, 2H, $-CH_2C_{carborane}$), 2.36 (m, 2H, $-CH_2C_{carborane}$), 2.47 (s, 6H, $-CH_3$), 4.02 (m, 4H, $-CH_2OTs$), 4.84 (br, 1H, $HC_{carborane}$), 5.05 (br, 1H, $HC_{carborane}$), 7.49 (d, 4H, ArH, J = 7.3 Hz), 7.79 (dd, 4H, ArH, J = 3.0 and 8.4 Hz). ^{11}B{^{1}H} NMR (CD_3COCD_3): δ −18.0 (s, 1B, B12−I/B9−I), −16.3 (s, 1B, B9−I/B12−I), −11.5 (m, 12B, B3, B4, B5, B6, B7, B11), −7.3 (s, 4B, B8, B10), −4.1 (s, 1B, B12−H/B9−H), −0.66 (s, 1B, B9−H/B12−H). ^{11}B NMR (CD_3COCD_3): δ −17.9 (s, 1B), −16.3 (s, 1B), −11.4 (m, 12B), −7.3 (d, 2B, J = 155.0 Hz), −4.0 (d, 1B, J = 149.8 Hz), −0.66 (d, 1B, J = 152.7 Hz). MS (HR-EI) for $C_{14}H_{27}O_3SIB_{10}$ (M^+). Calcd: m/z 510.1729. Found: m/z 510.1724.

Synthesis of 4a/4b (N5-I) [Strategy 2]. To a solution of **11a**/**11b** (1.18 g, 2.31 mmol) in DMF/acetone (1:1, 40 mL) was added dThd (1.68 g, 6.93 mmol) and potassium carbonate (960.0 mg, 6.94 mmol). The solution was stirred for 2.5 h at 40 °C and filtered. The filtrate was concentrated in vacuo, and the residue was added to water and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography using CH_2Cl_2/MeOH (10:1, R_f = 0.34) as the eluent to afford **4a**/**4b** (800 mg, 60%).

3-[5-(9-Bromo-*o*-carboran-1-yl)pentyl]thymidine and 3-[5-(12-Bromo-*o*-carboran-1-yl) pentyl]thymidine (12a/12b, N5-Br). To a solution of **4a**/**4b** (100 mg, 0.175 mmol) in 3 mL of CH_2Cl_2 was added a solution of NaBr (180 mg, 1.75 mmol) dissolved in 5 mL of water. The solvents were evaporated in vacuo to obtain an anhydrous residue. A solution of Herrmann's catalyst (41 mg, 25 mol %) in 1 mL of anhydrous DMF was added. The reaction mixture was stirred at 110 °C for 1 h. Following evaporation, the residue was added to water and extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$) and concentrated in vacuo, and the residue was purified by column chromatography to yield **12a**/**12b** (48 mg, 52%). R_f = 0.34 (CH_2Cl_2/MeOH, 10:1). ^{1}H NMR (CD_3COCD_3): δ 1.3−3.22 (m, 18H, BH), 1.31 (m, 4H, $-CH_2$), 1.56 (m, 8H, $-CH_2CH_2$), 1.83 (s, 6H, $-CH_3$), 2.23 (m, 4H, H-2′), 2.36 (m, 4H, $-CH_2C_{carborane}$), 3.77 (m, 4H, H-5′), 3.86 (m, 4H, $-CH_2N$), 3.92 (m, 2H, H-3′), 4.27 (s, 2H, −OH), 4.42 (s, 2H, −OH), 4.49 (d, 2H, H-4′, J = 2.9 Hz), 4.84 (s, 2H, $HC_{carborane}$), 6.34 (t, 2H, H-1′, J = 6.7 Hz), 7.83 (s, 1H, H-6). ^{11}B{^{1}H} NMR (CD_3COCD_3): δ −11.9 (m, 12B, B3, B4, B5, B6, B7, B11), −8.1 (s, 4B, B8, B10), −4.9 (s, 1B, B12−H/B9−H), −1.3 (s, 1B, B9−H/B12−H), 0.4 (s, 2B, B9−Br and B12−Br). ^{11}B NMR (CD_3COCD_3): δ −12.7 (m, 12B), −8.1 (d, 4B, J = 151.9 Hz), −4.9 (m, 2B), −0.72 (m, 2B). MS (HR-ESI) for $C_{17}H_{33}B_{10}BrN_2O_5$ [(M + Na)$^+$]. Calcd: m/z 557.2401. Found: m/z 557.2431.

3-[5-(9-[^{125}I]Iodo-*o*-carboran-1-yl)pentyl]thymidine and 3-[5-(12-[^{125}I]Iodo-*o*-carboran-1-yl)pentyl]thymidine (13a/13b, N5-^{125}I). An alkaline $Na^{125}I$ solution (10 μL, pH 8−11, 1 mCi, specific activity of 17 Ci [629 GBq]/mg) was diluted to 100 μL using deionized water. Compound **4a**/**4b** (10 mg, 0.017 mmol), dissolved in 200 μL of DMF, was transferred to a 3 mL conical reaction vial (MINUM-WARE). A solution of Herrmann's catalyst in 100 μL of DMF (10 mol %) was added to the reaction vial. To this reaction mixture was added dropwise 10 μL (100 μCi) of the diluted alkaline $Na^{125}I$ solution. The reaction mixture was then heated at 110 °C for 1 h, cooled to room temperature, and filtered using a GHP Acrodisc 13-mm syringe filter to separate the catalyst from the reaction mixture. The filtrate was evaporated under an argon flow at 30 °C, and the residue was purified by a semipreparative RP-18 HPLC to yield **13a**/**13b** (**N5-^{125}I**; 3.2 mg, radiochemical yield = 8%, specific activity = 2.52 μCi/mg, radio chemical purity: > 98%). HPLC retention time = 25.50 min (RP-18 analytical HPLC, 1 mL flow rate, solvent system CH_3CN/H_2O, 50:50, isocratic elution).

X-ray Diffraction Studies. Crystals of **8a** and **8b** were obtained by diffusion crystallization using diethyl ether/pentane as the solvent system. Both data collections were carried out at −123 °C on a Nonius Kappa CCD diffractometer with Mo Kα radiation and a graphite monochromator. ϕ and ω scans were used for data collection with a frame width of 1.0°. Data integration was carried out with Denzo,[32] and an absorption correction and merging of the data were carried out with Sortav.[33,34] The structures were solved by the direct methods procedure in *SHELXS-97*.[35] Full-matrix least-squares refinements based on F^2 were performed in *SHELXL-97*,[35] as incorporated in the *WinGX* package.[36] For structure **8b**, there are four independent molecules in the asymmetric unit (Z' = 4), and these are labeled as A−D (Table 3 and Figure S2 in the Supporting Information). For each methyl group, the hydrogen atoms were added at calculated positions using a riding model with U(H) = 1.5U_{eq} (bonded carbon atom). The torsion angle, which defines the orientation of the methyl group about the C−C or Si−C bond, was refined. The remaining hydrogen atoms in each cluster were refined isotropically. Neutral-atom scattering factors were used and include terms for anomalous dispersion.[37] The values of the Flack parameters[38,39] for **8a** and **8b** are −0.05(1) and −0.042(7), respectively, indicating that the models for these structures are of the correct chirality. Additional crystallographic details are shown in Table 2.

Docking Studies. The homology model of hTK1 (PDB ID # 1W4R) was developed from the crystal structure of *Thermotoga maritima* Thymidine Kinase (*Tm*TK, PDB ID # 2QPO) using the alignment mode of *SWISS-MODEL*.[40] The sequence identity and similarity between hTK1 and *Tm*TK are 36% and 55%, respectively.[41] The obtained homology model was saved in pdb format, and hydrogen atoms were added using Accelrys Discovery Studio Visualizer v2.5. After the addition of hydrogen atoms, the pdb file was imported into *Surflex*, version 2.11[42] (Biopharmics LLC, San Francisco, CA), installed on a Dell optimplex GX 270 desktop computer with a Windows operating system environment, for the protomol generation. The default mode was chosen for docking. The obtained docking poses were visualized with Accelrys Discovery Studio Visualizer v2.5. *Surflex* does not have parameters for boron atoms. By default, *Surflex* treats such unrecognizable atoms as "funky atoms". However, this does not seem to affect significantly the accuracy of the docking studies with compounds containing carborane clusters.[43]

PTAs. Recombinant hTK1 was expressed and purified from the bacterial expression system BL21(DE3) pLysS with transformed vector pET-14b + hTK1), kindly provided by Dr. Staffan Eriksson, Biomedical Centre, Uppsala, Sweden, according to a procedures described previously.[28,44] dThd and the carboranylthymidine conjugates were dissolved in dimethyl sufloxide (DMSO; 5 mM concentration) and further diluted with water to produce stock solutions of concentrations (0.4 mM). The PTAs were carried out as described previously[28,44] with minor modifications. The reaction mixture contained 100 μM nucleoside and 100 μM ATP [with a small fraction of 0.13 μM [γ-^{32}P]ATP (Perkin-Elmer)], 25 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 125 mM KCl, 1 mM DTT, and 0.5 mg/mL bovine serum albumin. In all reactions, the final concentration of DMSO was set to 2%. The reaction mixture was incubated at 37 °C for 20 min in the presence of 135 ng of enzyme. Somewhat higher concentrations of enzyme were used than in previous studies[45] because this specific enzyme preparation appeared to be less active (441 nmol of thymidine monophosphate formed per minute and mg of hTK1). Following the incubation period, the enzyme was inactivated by heating for 2 min at 99 °C. The reaction mixture was centrifuged, and 2 μL sample portions were spotted on PEI−cellulose

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TLC plates (EMD Chemicals Inc.). The TLC plates were developed in a solvent system containing isobutyric acid/ammonium hydroxide/water (66:1:33) over a period of 6 h. The radiolabeled spots were visualized by placing the TLC plates on a BioMax XAR film (Kodak) overnight and developing the films using an X-ray film developer (Tiba M6B, series VI B Rapid processor; Commonwealth X-ray Inc., Cincinnati, OH). The spot intensities of the phosphorylated compounds were calculated by using Photoshop, and the values were compared with those of dThd.

Cellular Uptake Studies. The L929 (#CCL1, American Type Culture Collection, Manassas, VA) cell line was one of the first to be established in a 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 (#CCL1.3 L-M ATCC) was derived from the wild-type cell line L929. The L929 TK1(+) cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine (#25030, Invitrogen, Carlsbad, CA), and a 1% penicillin and streptomycin mixture (Penstrep, #15140, Invitrogen). The L929 TK1(−) cells were grown in media with 10% FBS, 1% L-glutamine, 1% Penstrep, and 10 mM 5-bromo-2′-deoxyuridine in order to inhibit reversion to the TK1(+) phenotype. Cells were grown in T-75 flasks (#430641, Corning, Corning, NY) and split after reaching 70−80% confluency for propagating the cell line.

Uptake experiments were carried out as follows: 48 h prior to the addition of the test compounds, L929 TK1(−) or TK1(+) cells were seeded at a density of 1×10^6 or 2.5×10^6 cells per T-75 flask, respectively, with six flasks for each cell type. When the cells reached approximately 90% confluency, the test compounds were added to the culture medium. Cells were seeded at different densities because TK1(−) cells grew faster than TK1(+) cells. Before treatment, the cell culture medium was removed and the cells were washed once with DMEM. After that, 12.5 mL of media containing 17.5 μM **N5** (**3**), **N5**-$^{79/81}$**Br** (**12a**/**12b**), or **N5**-^{127}**I** (**4a**/**4b**) was added to the cultures. A total of 24 h following incubation at 37 °C, the medium containing the compound was removed and the cells were washed twice with cold phosphate-buffered saline at pH 7.4. Cells were then trypsinized with 5 mL of 0.5% trypsin/ethylenediaminetetraacetic acid (#15400, Invitrogen) for 5 min, followed by the addition of 5 mL of DMEM with 10% FBS to neutralize the trypsin. Cells with media from two T-75 flasks were pooled into one 50 mL centrifuge tube, following which they were centrifuged (600*g*). After centrifugation, the trypsin-containing medium was decanted, the cell pellets were resuspended in 2 mL of serum-free DMEM, and the number of cells per tube was counted by means of a hemocytometer. Cells with media then were transferred to borosilicate free glass test tubes, heated to dryness in an oil bath at 105 °C, and then 1 mL of concentrated sulfuric acid was added to digest them for 2 h. Following this, 1 mL of 50% hydrogen peroxide was added to each tube to decolorize the digested material. The contents were transferred to 15 mL plastic centrifuge tubes, and the volume was adjusted to 4 mL by the addition of deionized water. Boron concentrations were determined by means of inductively coupled plasma optical emission spectroscopy (ICP-OES) using a spectrometer located at the Nanotech West Facility of The Ohio State University.

RESULTS AND DISCUSSION

Computational Studies. Human TK1 tolerates extensive modification at the N3 position of dThd,[19] and the differences in the surface areas and the molecular volumes between **3** and **13a**/**13b**, or their nonradioactive counterparts (**N5**-^{127}**I**, [**4a**/**4b**, Scheme 1], were relatively small. The molecular surface area and volume for **3** were 575.53 Å^2 and 1297.05 Å^3, respectively. The corresponding values for **4a** and **4b** were 630.77 and 630.83 Å^2, respectively, for molecular surface areas and 1379.36 and 1379.75 Å^3, respectively, for molecular volumes (see the Supporting Information for details related to calculations). Therefore, we hypothesize that the introduction of a single iodine to **3** may not significantly affect its phosphorylation by hTK1, and more importantly, its tumor-localizing properties. Docking studies of the triphosphate forms of **3** and **4b** with a homology model of hTK1 seem to confirm our hypothesis (Figure 1). The extensive conformational changes

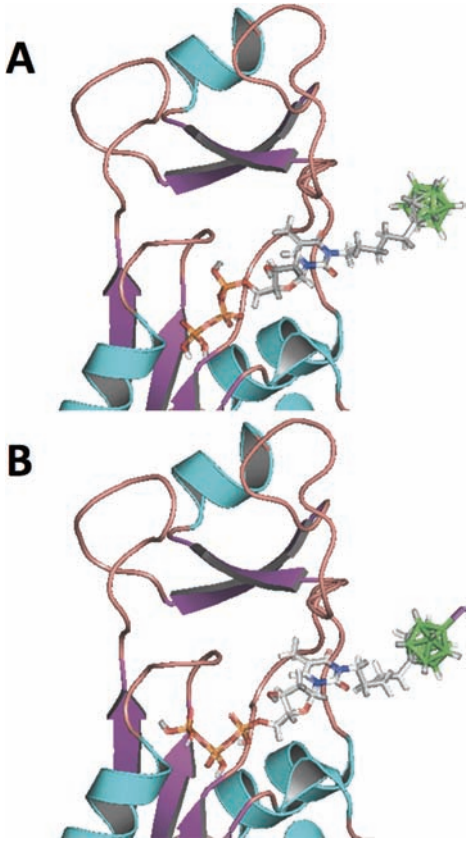

Figure 1. Docking poses of the 5′-triphosphate forms of **3** (A) and **4b** (B) in a homology model of hTK1 (PDB ID # 1W4R) that was developed from the crystal structure of *Tm*TK (PDB ID # 2QPO).

that TK1-like enzymes undergo as a result of ATP and/or dThd binding have been discussed in detail by Lavie et al.[46] The homology model of hTK1 was developed from an apo form of *Thermotoga maritima* Thymidine Kinase (*Tm*TK, PDB ID # 2QPO) and, thus, should represent an apo form of hTK1. The triphosphate forms of **3** and **4b** were chosen for docking because we have found that this modification produces realistic docking poses of the dThd scaffold within the hTK1 active site. As can be seen from parts A and B of Figure 1, the carborane clusters of both compounds are located outside the substrate binding pocket and monoiodination in **4b** does not generate significant differences in the dThd binding patterns between **3** and **4b**. Similar results have been observed previously in docking studies of other 3CTAs with varying substitution patterns at the carborane cluster in a hTK1 homology model.[47]

Chemistry. Theoretically, radioiodination of the *closo-o*-carborane cage can be achieved in three ways. The first method employs a "*closo*-cluster reconstruction" from *nido-o*-carborane via treatment with *n*-butyllithium (*n*-BuLi) followed by boron triiodide (BI_3) to generate iodinated *closo-o*-carborane.[48,49] The B-iodocarborane synthesized in this way has iodine either at B3 or at B6 (Figure 2). The second method is the direct introduction of iodine to the *closo-o*-carborane by means of a

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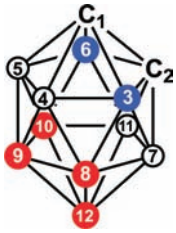


Figure 2. Atom numbering and electron density distribution in *closo-o*-carborane. Boron atoms shown in red are the most electron-rich, whereas the boron atoms shown in blue are the most electron-deficient.

Friedel–Crafts-type (electrophilic) halogenation using I_2 or ICl as iodine sources.[48,50,51] The electrophilic attack of positively charged species on *o*-carborane is regiospecific based on the charge distribution in the cluster.[48,50,51] Electrophilic halogenation occurs first at the most electron-rich boron atoms 9 and 12 (Figure 2), which are furthest away from both carbon atoms followed by the boron atoms 8 and 10.[48,50,51] Thus, reaction of *closo-o*-carboranes with halogenating species can result in mono-, di-, tri-, or tetraiodination depending on the applied reaction conditions and stoichiometry of halogenating species.[48,49] A disadvantage of both methods discussed above is that $^{125}I_2$, ^{125}ICl, or $B^{125}I_3$, which would be necessary for the synthesis of ^{125}I-labeled **3** (**N5-^{125}I**, **13a/13b**, Scheme 3), are not readily available from commercial sources.

The third method is a halogen/isotope-exchange reaction using a nonradioactive B-iodocarborane as the starting material that is treated with a nucleophilic radiohalogen source in the presence of a suitable catalyst.[6,8–10] The advantage of this method is that the radiohalogen source required for this strategy ($Na^{125}I$) is commercially available. This strategy was therefore chosen for the synthesis of **13a/13b**.

For the synthesis of **13a/13b** by means of isotope exchange, the nonradioactive precursor, **4a/4b**, must be synthesized first. Two methods were explored for its synthesis: (1) direct iodination of the carborane cage of **3** using either ICl or I_2 in the presence of a Lewis acid in a Friedel–Crafts halogenation strategy already discussed above and (2) initial monoiodination of unsubstituted *closo-o*-carborane with ICl followed by its functionalization to **4a/4b** using conventional synthetic organic methods.[28]

Direct Iodination of Preformed ***3*** *[Strategy 1].* A new strategy for the synthesis of **3** was applied (Scheme 1). The reaction of 6-heptynyl tosylate (**1**)[29] with decaborane ($B_{10}H_{14}$) in a biphasic mixture of toluene and the ionic liquid 1-butyl-3-methylimidazolium chloride ($bmim^+$ Cl^-)[52] yielded the *o*-carboranyl tosylate **2**[28] in 55% yield. The reaction of compound **2** with dThd in DMF/acetone in the presence of K_2CO_3 produced compound **3** in 56% yield, as described previously by Lunato et al.[28] Overall, the yields for both compounds **2** and **3** described here are comparable with those reported previously for the same compounds.[28] However, the synthesis of **3** is much shorter, far more convenient, and also safer than the procedure described previously involving the synthesis and isolation of an intermediate decaborane/acetonitrile complex.[28]

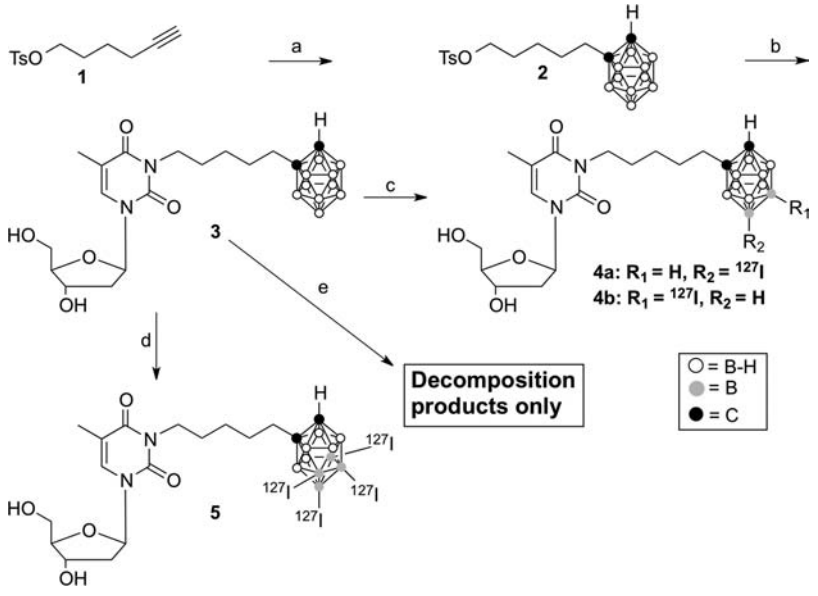
Several different reaction conditions were explored to optimize the synthesis of **4a/4b** via direct iodination of **3**, as shown in Scheme 1 and Table 1. The treatment of **3** with 5 equiv of ICl in the absence of Lewis acid ($AlCl_3$) at room temperature for 1 h in CH_2Cl_2 proved to be detrimental to the nucleoside scaffold, resulting in complete decomposition. When **3** was treated with 1 equiv of ICl in the presence of

Table 1. Reaction Conditions To Control the Degree of Iodination by Strategy 1

	% in reaction mixtures[a]					
reactions (Scheme 1)	dec prod	**3** (recov)	**4**	**N5-I_2**	**N5-I_3**	**5**
c	~10	~45	~40	~5	0	0
d	~35	0	0	0	~10	~55
e	100	0	0	0	0	0

[a]Estimations based on TLC and ^{1}H NMR analyses of reaction mixtures.

Scheme 1[a]



[a]Reagents and conditions: (a) $B_{10}H_{14}$, $(bmim)^+Cl^-$, toluene, 120 °C, 10 min; (b) dThd, K_2CO_3, DMF/acetone (1:1), 40 °C, 24 h; (c) ICl, $AlCl_3$, DCM, 0 °C, 2 h; (d) ICl, $AlCl_3$, DCM, 40 °C, 48 h; (e) ICl, DCM, rt, 1 h.

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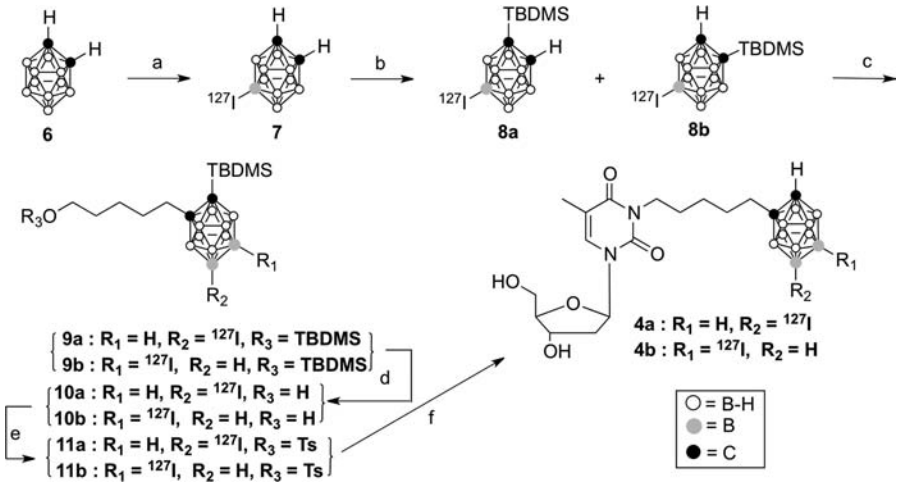
Scheme 2[a]



[a]Reagents and conditions: (a) ICl, DCM, 40 °C, 5 h; (b) *n*-BuLi, TBDMSCl, THF, 66 °C, 12 h; (c) *n*-BuLi, 5-(*tert*-butyldimethylsilyloxy)pentyl 4-methylbenzenesulfonate; THF, 66 °C, 12 h; (d) (i) TBAF, THF, −78 to +4 °C, 30 min; (ii) 10% methanolic HCl, 4 °C, 30 min; (e) TsCl, Et_3N, DMAP, 0 °C to rt, 7 h; (f) dThd, K_2CO_3, DMF/acetone (1:1), 40 °C, 2.5 h.

10 equiv of $AlCl_3$ in CH_2Cl_2 at 0 °C for 2 h, the reaction mixture contained 40% of **4a**/**4b**, ∼45% of unreacted **3**, and minor quantities of diiodinated **3** (**N5-I$_2$**) and decomposition products (Table 1). The reaction of **3** with 5 equiv of ICl and 15 equiv of $AlCl_3$ at 40 °C in CH_2Cl_2 for 48 h resulted in the formation of ∼55% of compound **5** (**N5-I$_4$**) along with smaller quantities of triiodinated **3** (**N5-I$_3$**) and decomposition products in the reaction mixture (Table 1). Preliminary analytical data indicated that the decomposition products may include nucleobases and pyranosyl nucleosides (data not shown). In general, compounds **4a**/**4b**, **5**, **N5-I$_2$**, and **N5-I$_3$** were crudely separated from decomposition products (primarily nucleobases) and inorganic impurities by column chromatography followed by complete purification via semipreparative HPLC. When $AlCl_3/I_2$ instead of $AlCl_3$/ICl was used in these reactions, overall similar product ratios were observed (data not shown).

*Initial Monoiodination of o-Carborane followed by Functionalization to Compounds **4a/4b** [Strategy 2].* Scheme 2 describes the synthesis of **4a**/**4b** according to strategy 2. The reaction of *o*-carborane with ICl in the absence of any Lewis acids under reflux conditions in CH_2Cl_2 furnished 9-iodo-*o*-carborane (**7**)[30,31,53] in 70% yield without producing detectable amounts of di-, tri-, or tetraiodinated *o*-carboranes. This monoiodination of *o*-carborane was operationally very convenient, and **7** was the *only* product obtained even when an excess of ICl was used. It should be noted that, in the absence of Lewis acid, I_2 did not cause any iodination of *o*-carborane (data not shown), whereas both ICl and I_2 were far more reactive in the presence of $AlCl_3$, causing even tetraiodination of **3**, as discussed above.

Deprotonation of the carboranyl C−H of **7** by *n*-BuLi, followed by the reaction with TBDMSCl, resulted in the formation of a mixture of structural isomers **8a** and **8b** in 66% overall yield with iodine at either B9 or B12 (Scheme 2 and Figure 2). This protection of one of the carboranyl carbon atoms of **7** with the TBDMS group was carried out to guarantee the subsequent monofunctionalization of carboranyl carbon.[54,55] Unfortunately, the column chromatographic separation of larger quantities of **8a** and **8b** from their mixture was very tedious even though they have distinct R_f values (see the Experimental Section). The same was true for all other compound mixtures described in Schemes 1−3 because of very close R_f values (see the Experimental Section). Therefore, we decided to carry out all followup reactions, optimization of the halogen/isotope reactions, and all biological evaluations with isomeric mixtures because this would not affect most of the major objectives of this study. As already discussed, the carborane cage of 3CTAs may be located outside the hTK1 substrate-binding site. Therefore, the regioselectivity of monoiodination (B9−I vs B12−I) should not affect the phosphorylation by hTK1 significantly (Figure 1). It was, however, important to confirm the formation of structural isomers with iodine and the TBDMS group in *meta* and *para* orientations, respectively, and to ensure that nucleophilic displacement/exchange of iodine did not occur after the treatment of 9-iodo-*o*-carborane with *n*-BuLi (Figure 4). Therefore, a small quantity of **8a**/**8b** was separated by silica gel chromatography for a thorough analysis by ^1H, ^{13}C, and ^{11}B NMR, X-ray crystallography (Figure 4), and HRMS.

In the next reaction step, the carboranyl C−Hs of isomeric **8a**/**8b** were deprotonated by *n*-BuLi followed by the reaction with 5-(*tert*-butyldimethylsilyloxy)pentyl 4-methylbenzenesulfonate to afford **9a**/**9b** in 66% yield. Complete desilylation of **9a**/**9b** was accomplished by the reaction with TBAF/THF for 30 min at 0 °C followed by the addition of 10% methanolic HCl[56] and continued stirring for 30 min at room temperature to obtain **10a**/**10b** in 76% yield. The reaction with TBAF alone resulted in complete deprotection of the C-TBDMS group of **9a**/**9b** but only in the partial removal of the O-TBDMS group. The complete cleavage of the O-TBDMS group by TBAF using longer reaction times, higher temperatures, and/or higher equivalents (2.2−2.5) of TBAF was not explored because there was a possibility of degradation of the *closo*-*o*-carborane cage to *nido*-carborane.[48,57] On the other hand, the reaction of **9a**/**9b** with 10% methanolic HCl[56] alone for 30 min at room temperature led to the complete removal of the O-TBDMS group, whereas the C-TBDMS remained unaffected. The free hydroxyl groups of **10a**/**10b** were functionalized to the corresponding tosylates (**11a**/**11b**) in 70% yield, which were then subjected to treatment with dThd in the presence of K_2CO_3 in

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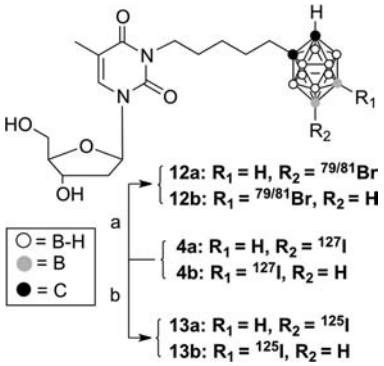
DMF/acetone (1:1) to afford **4a**/**4b** in 60% yield after purification by column chromatography. All compounds described in Schemes 1 and 2 were analyzed by ^{1}H, ^{13}C, and ^{11}B NMR and HRMS.

*Halogen/Isotope-Exchange Reactions with **4a**/**4b**.* In order to optimize the reaction conditions for the final isotope-exchange reaction (Scheme 3, reaction b), compounds **4a**/**4b**

Scheme 3[a]



[a]Reagents and conditions: (a) $Na^{79/81}Br$, Herrmann's catalyst, DMF, 110 °C, 1 h; (b) $Na^{125}I$, Herrmann's catalyst, DMF, 110 °C, 1 h.

were initially subjected to a halogen-exchange reaction using 10 equiv of NaBr in the presence of Herrmann's catalyst (25 mol %) in DMF at 110 °C for 1 h (Scheme 3, reaction a). This resulted in 100% halogen exchange to afford **N5-$^{79/81}$Br** (**12a**/**12b**) in 52% yield following column chromatographic purification, as indicated by ^{1}H and ^{11}B NMR and mass spectrometry. Commercial $Na^{125}I$ is available in the form of an aqueous alkaline solution (pH 8−11). Strong bases such as the methoxide ion, ammonia, alkylamines, and piperidine are known to degrade *closo-o*-carboranes into the corresponding *nido*-carboranes.[48,57] In order to measure the possible extent of degradation of **4a**/**4b** during the isotope-exchange reaction using an alkaline solution of $Na^{125}I$, **4a** and **4b** were also treated with 0.5 equiv of NaBr and 5 mol % NaOH in DMF at 110 °C for 1 h. ^{1}H and ^{11}B NMR spectroscopy did not indicate the formation of measurable quantities of the corresponding *nido* species during this pilot reaction. Additional pilot reactions for optimization of halogen/isotope exchange with different iodo-*o*-carboranyl compounds are described in the Supporting Information.

The isotope-exchange reaction of **4a**/**4b** was carried out with $Na^{125}I$ in the presence of 10 mol % Herrmann's catalyst in DMF at 110 °C for 1 h followed by semipreparative HPLC purification. The reaction was also monitored by radio-TLC (Supporting Information). Both the analytical HPLC UV chromatogram and the radioanalytical trace of the purified product (**13a**/**13b**) displayed split peaks, which is consistent with the presence of two structural isomers (Figure 3). The specific activity, radiochemical yield, and radiochemical purity of **13a**/**13b** were 2.55 μCi/mg, 8%, and 98%, respectively. The specific activity obtained may be sufficient for preliminary in vitro uptake studies of **13a**/**13b**.[58] The primary intention of this isotope-exchange reaction was proof-of-concept, and no attempts were made to improve the specific activity and radiochemical yield. This could be accomplished by varying the reaction conditions and stoichiometries of the reagents. Furthermore, the specific activity of the final product could be improved significantly by employing a "reversed" halogen exchange (B−$^{79/81}$Br to B−^{125}I) followed by the chromatographic separation of the halogenated species. Preliminary

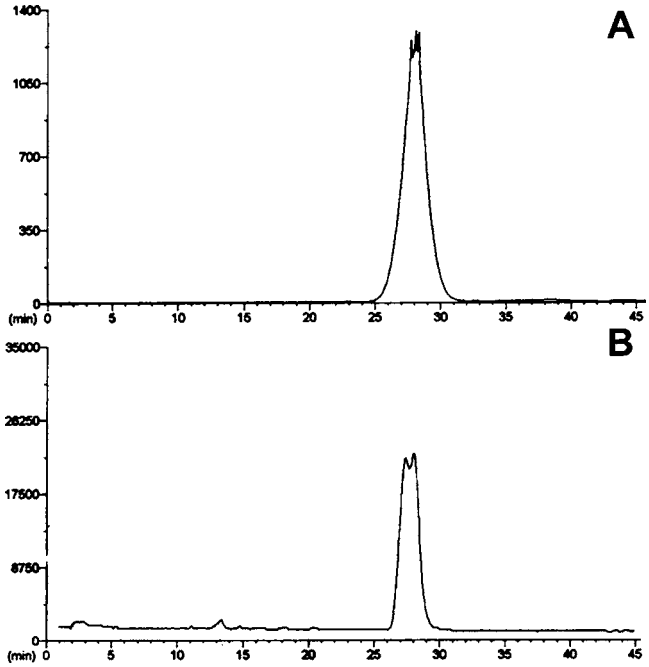


Figure 3. HPLC analysis of **N5-^{125}I** (**13a**/**13b**): (A) radioanalytical trace; (B) UV chromatogram. Split peaks are likely due to the presence of two structural isomers.

HPLC studies indicated that it may be possible to separate **N5-^{127}I** (**4a**/**4b**) from **N5-$^{79/81}$Br** (**12a**/**12b**) (see the Supporting Information).

X-ray Crystallography. X-ray crystallography confirmed the formation of the structural isomers **8a** and **8b** with iodine and the TBDMS group in *meta* and *para* orientations, respectively (Figure 4). Compound **8a** crystallized with one molecule in the asymmetric unit ($Z' = 1$), whereas compound **8b** was obtained as a collection of four slightly different rotamers ($Z' = 4$ and labeled as A−D; see Table 2 for crystallographic details and Figure S2 in the Supporting Information for structures). The four rotamers of **8b** have different torsion angles for C5−Si1−C1−C2, which are −131.8(2)° (A), −140.6(2)° (B), 138.5(2)° (C), and 142.1(2)° (D). Interestingly, the C5−Si1−C1−C2 torsion angle found for **8a** was 6.3(2)°. Structure **8a** does not show any significant I···I halogen bond-type interactions, whereas structure **8b** does form two of these. Both structures show significant C_{carb}−H···I−B interactions (see Table 3). These types of intermolecular interactions are similar to those observed by Puga et al. in the crystal lattices of iodinated *o*-carboranes.[53] Table 3 lists selected bond lengths in **8a** and in all four rotamers of **8b** along with the C_{carb}−H···I−B and I···I interactions. The bond lengths found for both crystal structures were in agreement with those reported by Puga et al. for similar compounds.[53]

Biological Studies. *PTAs.* In order to evaluate their hTK1 substrate characteristics, PTAs[28,44] with recombinant hTK1 were carried with **N5** (**3**), **N5-^{127}I** (**4a**/**4b**), **N5-$^{79/81}$Br** (**12a**/**12b**), and **N5-I_4** (**5**). The results are shown in Figure 5 and Table 4. The phosphorylation rate of dThd, being a natural substrate of hTK1, is set to 100%, and the phosphorylation rates of the test compounds are expressed relative to that of dThd. They indicate that substitution of hydrogen in the carborane cluster of **3** by either iodine (**4a**/**4b**) or bromine (**12a**/**12b**) only had minimal effects on the phosphorylation rates (38.0% for **3** vs 36.9% for **12a**/**12b** vs 29.6% for **4a**/**4b**). The observed phosphorylation rate for **3** was comparable to that reported previously for the same compound.[28]

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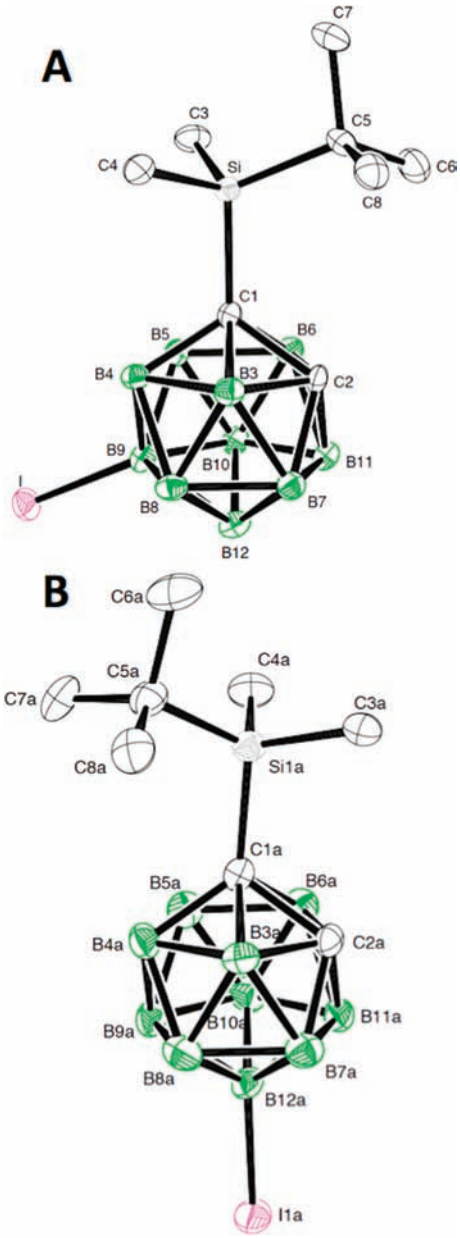

Figure 4. ORTEP plots of **8a** and **8b** drawn with 50% probability displacement ellipsoids. The hydrogen atoms are omitted for clarity. Only one of the four molecules in the asymmetric unit, molecule A, is shown for structure **8b**.

The moderate decrease in phosphorylation of **4a**/**4b** and **12a**/**12b** compared with **3** substantiates our hypothesis that the carborane cage of 3CTAs is located far away from the site of phosphorylation and that the introduction of one iodine or bromine at the carborane cage did not significantly affect hTK1 substrate characteristics. On the other hand, the introduction of four iodine atoms at the carborane cage, as in compound **5**, affected binding to hTK1, resulting in markedly decreased phosphorylation, which could not be accurately determined (Figure 5). The chromatogram shown in Figure 5 displays additional spots for two unidentified ^{32}P-containing compounds (b) that were formed during phosphoryl transfer or the following workup. It is conceivable that these spots were the monophosphates of the *nido*-carboranyl forms of **4a**/**4b** and **12a**/**12b** because halogenated carboranes

Table 2. Crystallographic Data for 8a and 8b

	8a	8b
formula	$C_8H_{25}B_{10}ISi$	$C_8H_{25}B_{10}ISi$
fw	384.37	384.37
space group	*Cc* (No. 9)	$Pca2_1$ (No. 29)
a, Å	6.8264(1)	13.6530(1)
b, Å	20.6488(3)	19.5285(1)
c, Å	12.5390(2)	27.7225(2)
β, deg	90.346(1)	
volume, Å^3	1767.43(5)	7391.45(8)
Z	4	16
density (calcd), g/cm^3	1.444	1.382
μ, cm^{-1}	18.59	17.78
cryst size, mm^3	0.04 × 0.12 × 0.38	0.19 × 0.19 × 0.27
final *R* indices $[I > 2\sigma(I)]^a$	R1 = 0.0238, wR2 = 0.0421	R1 = 0.0303, wR2 = 0.0460
R indices (all data)^a	R1 = 0.0316, wR2 = 0.0440	R1 = 0.0538, wR2 = 0.0489

^{a}R1 = $\sum||F_o| - |F_c||/\sum|F_o|$. wR2 = $[\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$.

Table 3. Selected Bond Lengths (Å) and Selected Intermolecular Interactions for 8a and the Four Rotamers of 8b (A–D)

selected bonds^a	8a^a	8b (A)^b	8b (B)	8b (C)	8b (D)
B–I	2.182(3)	2.175(4)	2.178(4)	2.177(4)	2.175(4)
C1–Si	1.941(3)	1.937(3)	1.943(3)	1.930(3)	1.940(3)
C1–C2	1.664(4)	1.667(4)	1.664(4)	1.664(4)	1.661(4)
selected intermolecular interactions	**8a^a**	**8b (A)^b**	**8b (B)**	**8b (C)**	**8b (D)**
C_{carb}–H·····I–B	3.37	3.17–3.38			
I·····I	NA^c	3.70 and 4.10			

^{a}The *Mercury* program, version 2.4,[59] of the Cambridge Crystallographic Data Centre was used to calculate these intermolecular distances. ^{b}See Figure 4 for the numbering of **8a** and **8b** (A). Numbers for **8b** (B), **8b** (C), and **8b** (D) are the same as that for **8b** (A). ^{c}NA: not applicable.

may be somewhat more susceptible to *nido* formation than non-halogenated carboranes.[48]

Cellular Uptake Studies. Uptake of **3**, **12a**/**12b**, and **4a**/**4b** by L929 TK1(+) cells was quantified by determining the boron concentrations by means of ICP-OES (Table 4). These were 2, 1.4, and 1.8 times greater than those of L929 TK1(−) cells, indicating that TK1 activity may play a major role in the in vitro uptake of these compounds. However, there was no obvious correlation with the hTK1 phosphorylation rates of these compounds. We do not have an explanation for the uptake data of **4a**/**4b** in both L929 TK1(+) and TK1(−) cells, which were higher than those of **3** and **12a**/**12b** in the same cell lines. Compound **5** was not evaluated in the cellular uptake studies because of lack of material. However, cellular uptake of boronophenylalanine (BPA), a drug used clinically for BNCT,[60] did not differ significantly (*P* value = 0.563) between L929 TK1(+) and L929 TK1(−), as determined by a two-sample *t* test. This was expected because BPA is a boron-containing amino acid that is not a substrate of TK1. Uptake of **3**, **12a**/**12b**, and **4a**/**4b** in L929 TK1(−) cells was also very high, indicating that other factors, such as compound lipophilicity, causing nonspecific retention in cellular membranes, or nucleoside/nucleotide influx and/or efflux, may have played important roles in the uptake and retention of these compounds.

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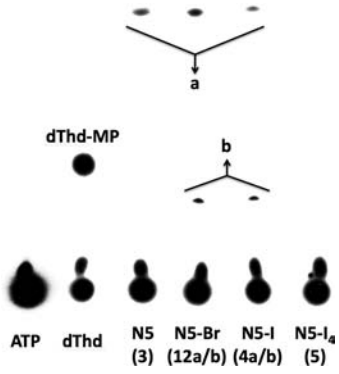

Figure 5. Phosphorylation of dThd, **N5** (**3**), **N5**-$^{79/81}$**Br** (**12a**/**12b**), **N5**-^{127}**I** (**4a**/**4b**), and **N5**-^{127}**I**$_4$ (**5**) by recombinant TK1. Assay products were separated by PEI−cellulose TLC. (a) Monophosphates of **N5** (**3**) **N5**-^{127}**I** (**4a**/**4b**), and **N5**-$^{79/81}$**Br** (**12a**/**12b**). (b) Unidentified phosphorylation products with relative phosphorylation rates of 14 ± 0.7% (**N5**-^{127}**I** lane) and 25 ± 1.1% (**N5**-$^{79/81}$**Br** lane).

Table 4. Human TK1 Phosphorylation Rates of N5 (3), N5-^{127}I (4a/4b), and N5-$^{79/81}$Br (12a/12b), Relative to That of dThd, and in Vitro Uptake Data of N5 (3), N5-^{127}I (4a/4b), N5-$^{79/81}$Br (12a/12b), and BPA[a]

compounds	phosphorylation rate (%)	uptake (μg of B/10^9 cells) L929 TK1(+)	L929 TK1(−)
dThd	100	nd	nd
N5 (**3**)	38.0 ± 2.2	77.6 ± 6.2 [d]	38.6 ± 3.3
N5-$^{79/81}$**Br** (**12a**/**12b**)	36.9 ± 0.4	76.0 ± 9.0 [b]	54.0 ± 5.3
N5-^{127}**I** (**4a**/**4b**)	29.6 ± 0.5	118.2 ± 24.3 [c]	65.5 ± 5.3
BPA[e]	nd[e]	48.0 ± 8.9	42.1 ± 13.6

[a]Data represent the means of four replicates ± SD for phosphorylation rates, three replicates ± SD for cellular uptake studies with **3**, **12a**/**12b**, **5**, and BPA, and six replicates ± SD for cellular uptake studies with **4a**/**4b**. [b]$P < 0.05$. [c]$P < 0.01$. [d]$P < 0.001$, compared with L929 TK1(−). [e]BPA: boronophenylalanine. nd: not determined. BPA is not a nucleoside analogue. Hence, phosphorylation rate determination was not applicable.

SUMMARY AND CONCLUSIONS

Both synthetic strategies explored for the synthesis of **4a**/**4b** were successful. However, even under optimized reaction conditions, strategy 1 generated a complex reaction mixture containing decomposition products, diiodinated material, and substantial quantities of unreacted **3**. This was a major disadvantage of strategy 1 because it necessitated the purification of **4a**/**4b** not only by conventional column chromatography but also by semipreparative HPLC. On the other hand, strategy 1 also generated the tetraiodinated compound **5**. PTAs indicated that this compound might not be a good substrate of hTK1, presumably because of increased bulkiness at the carborane cage. However, tetraradioiodination potentially may be an important approach for other *closo*-carboranyl radiopharmaceuticals, especially those that might be used for cancer treatment. From this perspective, it is important to develop a general strategy for the tetraiodination of *closo*-carboranyl biomolecules, as reported in this study. In general, strategy 2 allowed for a more controlled synthesis of **4a**/**4b**. Potentially, separation of the isomers **4a** and **4b** is also possible using this strategy on the level of compounds **8a** and **8b**. Radiohalogenation of **4a**/**4b** to obtain **13a**/**13b** was successful. Further optimization of the reaction conditions should result in improved specific activity and radiochemical yield of **13a**/**13b**. To the best of our knowledge, this is the first report of halogen- and isotope-exchange reactions at a B-halocarborane, which is conjugated to a complex biomolecule such as dThd. Noteworthy is the observed stability of the B−I bond under a variety of reaction conditions. In general, the synthetic technology developed at the examples of **12a**/**12b** and **13a**/**13b** may pave the way for the synthesis of a wide range of carborane cage radiohalogenated therapeutics and diagnostics.

Only small differences in hTK1 phosphorylation rates were found between **3**, **12a**/**12b**, and **4a**/**4b**. This supports our original assumption that monohalogenation of **3** will not drastically change its hTK1 substrate characteristics. In contrast, tetraiodination of **3**, as in compound **5**, notably decreased the hTK1 phosphorylation rate, indicating that there is a limit for size increase at the carborane cage. These findings also reinforce our initial hypothesis that mono(radio)iodination at the carborane cage of **3** is at least an appropriate tool to study the real-time pharmacokinetics of this 3CTA. The phosphoryl transfer rates obtained for **3**, **12a**/**12b**, and **4a**/**4b** were supported by a higher uptake in L929 TK1(+) than in L929 TK1(−) cells. However, uptake of all three compounds in L929 TK1(−) cells was also substantial, indicating that mechanisms other than KMT, such as passive diffusion, may have contributed to their accumulation.

ASSOCIATED CONTENT

Supporting Information

Additional data, including additional general experimental information, ^{13}C NMR chemical shifts, supporting synthetic, computational, chromatographic, and radioanalytical studies, as well as detailed X-ray crystallographic and spectroscopic information are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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