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Improved synthesis of MC4-PPEA and the biological evaluation of its hydroxymethyl derivative

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ABSTRACT

Nicotinamide phosphoribosyltransferase (Nampt) is an intriguing target for the treatment of many diseases, including cancer. Previously, our group demonstrated that carborane clusters may be used to increase the potency of small molecule inhibitors of Nampt over other, similarly sized organic moieties. Herein, we report a greatly improved, gram-scale synthesis of our most potent agent: 1-(4′-(*trans*-3″-(3‴-pyridyl) acrylamide)butyl)-1,7-dicarba-closo-dodecaborane (MC4-PPEA). Additionally, the carborane moiety of the molecule has been modified with a hydroxymethyl functional group to allow for its covalent attachment to targeted prodrugs, the synthesis of which are underway. Using cell viability assays, we demonstrate that this new derivative exhibits low, to mid-nanomolar potencies against human breast cancer cell lines in vitro.

There is a need for new antitumoral drugs which are more efficacious against a wide range of cancers. This need is even greater for advanced or recurrent cancers which too often respond poorly to current treatments. A promising new target for the treatment of cancer is nicotinamide phosphoribosyltransferase (Nampt), the first and rate limiting enzyme in the mammalian NAD^+ recycling pathway. This enzyme catalyzes the conversion of nicotinamide to nicotinamide mononucleotide. NAD^+ is a vital cofactor used throughout cellular respiration and thus, Nampt plays a key role in regulating cellular metabolism. Nampt has been shown to be upregulated in many/most cancers[1–8] and this overexpression is highest in aggressive and refractory cancers.[9,10] FK866 was the first known small molecule inhibitor of Nampt and has been investigated in Phase I/II clinical trials against several cancers.[11,12]

Our research group recently reported a small family of carborane-containing Nampt inhibitors which exhibit low, to sub-nanomolar potencies against four human cancer cell lines in vitro.[13] Owing to the high boron content of carboranes and the extremely high thermal neutron cross sectional area of the isotope boron-10, these clusters have been extensively studied as building blocks for the development of agents for boron neutron capture therapy (BNCT).[14,15] Apart from this area, there is an increasing interest in the use of carboranes as ridged, hydrophobic moieties for drug discovery. Several excellent reviews and books have been published in this area.[16–20] Our most potent derivative, MC4-PPEA (**4**), exhibited a 10-fold higher activity and an approximately 100-fold greater inhibition of Nampt in vitro when compared with the lead molecule FK866.[13] We demonstrated that the carborane clusters incorporated into the drug structure increased the potency of the new molecules over other purely organic groups, such as phenyl, or adamantly moieties. We also compared the potencies for molecules incorporating *ortho*-, *meta*-, or *para*-carborane and found that the molecule containing the *m*-isomer exhibited the highest potency, suggesting that strong carborane–enzyme interactions are formed, possibly through the formation of dihydrogen bonds.[21] Recent results obtained through the National Cancer Institute's Developmental Therapeutics Program confirm that **4** is significantly more potent than FK866 in the 60 human cancer cell lines employed by the NCI-60 screen.[22]

In the present study, we have optimized the synthesis of **4** using a new route towards the intermediate amine and a new coupling strategy, significantly improving the scalability, yield and purity of the final product. We have also synthesized a new derivative **11** bearing a hydroxymethyl group appended to the *meta*-carborane moiety. This group will serve as a reactive moiety for the future covalent attachment of the molecule, through various

Abbreviations: Nampt, nicotinamide phosphoribosyltransferase; MC4-PPEA, 1-(4′-(*trans*-3″-(3‴-pyridyl) acrylamide)butyl)-1,7-dicarba-closo-dodecaborane; BNCT, boron neutron capture therapy; BOP, benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; HMPA, hexamethylphosphoramide; PAC, *trans*-3-(3′-pyridyl)acryloyl chloride; IC_{50}, half maximal inhibitory concentrations; MTT assay, microculture tetrazolium assay.

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hydrolysable linkers, to cancer-associated targeting vectors; efforts which are now underway in our group.

Our initial attempts to synthesize a conjugate of **4** focused on the substitution of the nitrogen atom of the pyridyl moiety. However, preparation of an oxymethylenecarbonyl derivative, similar to that found in the Nampt inhibitor GMX1777, suffered from very poor yields.[23] Furthermore, the conjugate produced was hydrolytically unstable. Attempts were also made to prepare an ester derivative from a carboxyl group bound to the unsubstituted carbon atom on the carborane cluster of **4** without success. New efforts to introduce other reactive groups for producing conjugates with enhanced potency are currently underway.

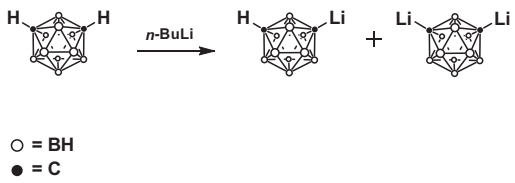
The potency of the new hydroxymethyl derivative was tested using cell viability assays in two human breast cancer cell lines, as well as a non-cancerous, chemically transformed breast epithelial cell line which expresses Nampt.

In our initial report, we prepared 40 mg of **4** using a total of four synthetic steps in seven percent yield overall.[13] Here, we have improved the overall synthetic yield to twenty one percent, while increasing the scale of the reactions by a factor of 20. The synthesis of **4**, as previously described,[13] and the sequence of reactions leading to optimized and improved synthesis of **4** are depicted in Scheme 1.

To synthesize **4**, *m*-carborane was first activated using *n*-butyllithium, allowing for a metalation type reaction on the weakly acidic C–H proton to create nucleophilic sites.[16] These nucleophiles can be used to prepare a wide range of carborane based derivatives (Scheme 2).[16]

The lithiated *m*-carborane was then reacted with 1-chloro-4-iodobutane, producing 1-(4-chlorobutyl)-*m*-carborane (**1a**)[13] as a colorless oil with a yield of 51%. The di-substituted side product (**1b**) (yield of 32%) and some starting material were also recovered. Both mono- and di-substituted products were isolated using column chromatography on silica gel. The reactions following this step involved manipulation of the terminal end of the substituted alkyl group placed on the carborane cage and did not involve the cage itself.

The chlorine on compound **1a** was exchanged with an azide using sodium azide in an S_N2 type reaction to afford 1-(4-azidobutyl)-*m*-carborane (**2**) as a colorless oil with a yield of 100%. Compound **2** was reduced using palladium on activated carbon under a hydrogen atmosphere to afford aminobutyl-*m*-carborane (**3**) as a yellow oil with 60% yield. The product **4** was synthesized

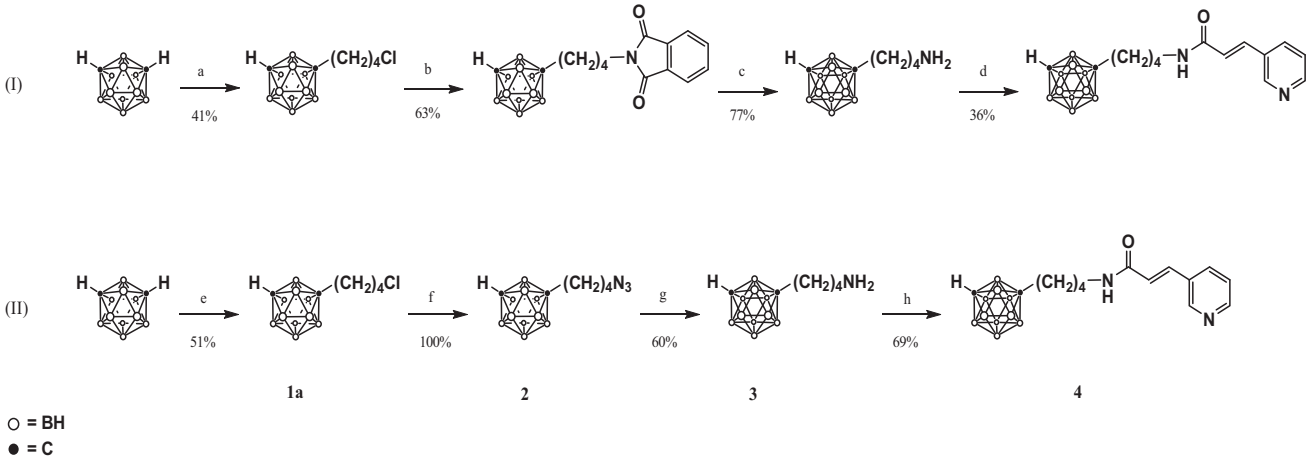


Scheme 2. Metalation of *m*-carborane using *n*-butyllithium.

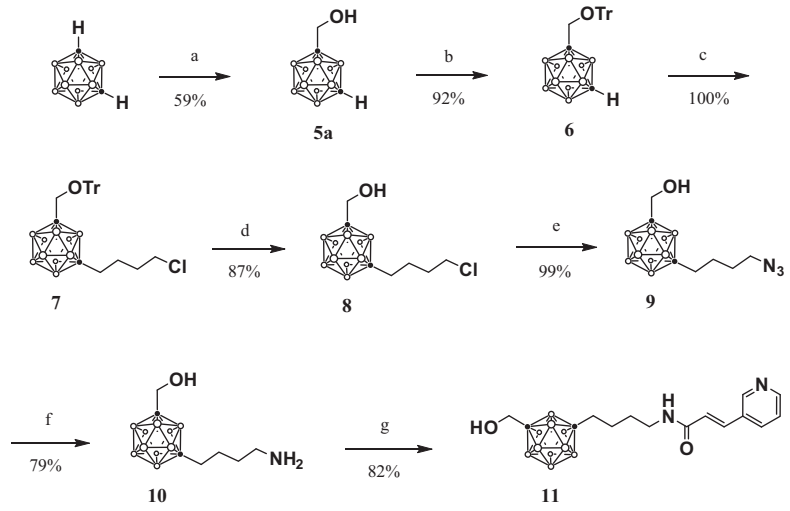
by converting *trans*-3-(3′-pyridyl)acrylic acid to the acid chloride, making it more susceptible to react with the amine without the need for a coupling agent, such as the benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) used previously. In a one-pot, two-step reaction, *trans*-3-(3′-pyridyl)acrylic acid was treated with thionyl chloride; DMF was used as a catalyst to achieve *trans*-3-(3′-pyridyl)acryloyl chloride. Sulfur dioxide and hydrochloric acid gas were produced as byproducts and removed in vacuo. The product was redissolved in THF and the remaining acid was captured using Hünig's base. A THF solution of **3** was then added drop-wise and the reaction mixture was allowed to stir overnight. This reaction afforded 1-(4′-(*trans*-3″-(3‴-pyridyl)acrylamido)butyl)-1,7-dicarbadodecaborane (**4**) as an orange foam with 69% yield, a significant improvement over the 36% yield previously observed.[13]

One reason that the use of BOP as a coupling reagent was avoided was owing to the challenge of isolating the product from the many byproducts that result from that reaction. One such byproduct was hexamethylphosphoramide (HMPA) which is a polar aprotic solvent having a high boiling point (232.5 °C). HMPA is known to be carcinogenic and its complete removal was not possible using flash chromatography. Due to the presence of HMPA, the product stayed in solution as a thick yellow oil. Previously, it was necessary to utilize high pressure liquid chromatography (HPLC) to recover **4** as a pure solid product. The purification of product using HPLC was time consuming and was not practical at the gram scale. Under the present conditions, the pure product can be obtained using flash chromatography on silica gel.

The synthetic route for hydroxymethyl functionalized **11** is depicted in Scheme 3. An acidic proton on one of the carbon atoms of *m*-Carborane was removed using *n*-butyllithium and the lithiated *m*-carborane was then reacted with paraformaldehyde.[24,25] After quenching the reaction with 2 N $HCl_{(aq)}$, 1-(hydrox-



Scheme 1. Previously reported synthetic pathway (I)[13] and improved procedure (II) for compound **4**. Reagents and conditions: (a) *n*-BuLi, THF, 4 h, rt, 1-chloro-4-iodobutane, overnight, rt. (b) Potassium phthalimide, DMF, reflux, overnight. (c) Hydrazine, EtOH, 4.5 h reflux. (d) DMF, BOP, Et_3N, *trans*-3-(3′-pyridyl)acrylic acid, overnight rt. (e) *n*-BuLi, THF, 0 °C–rt, 1-chloro-4-iodobutane, −78 °C to rt, overnight. (f) NaN_3, NaI (cat.), DMF, 70 °C, overnight. (g) Pd/C, H_2, MeOH, overnight, rt. (h) *trans*-3-(3-Pyridinyl)-2-propenyl chloride, DIPEA, THF, −78 °C to rt, overnight.



Scheme 3. Synthesis of hydroxymethyl functionalized MC4-PPEA (**11**). Reagents and conditions: (a) *n*-BuLi, benzene/Et_2O, 0 °C–rt, paraformaldehyde, 0 °C–rt, 2 N HCl. (b) Trityl chloride, DIPEA, DCM, rt. (c) *n*-BuLi, THF, 0 °C–rt, 1-chloro-4-iodobutane, −78 °C to rt, 2 h. (d) HCl (concd), MeOH:$CHCl_3$, rt. (e) NaN_3, NaI (cat.), DMF, 70 °C. (f) PPh_3, H_2O, THF, rt. (g) *trans*-3-(3-Pyridinyl)-2-propenyl chloride, DIPEA, THF, −78 °C to rt.

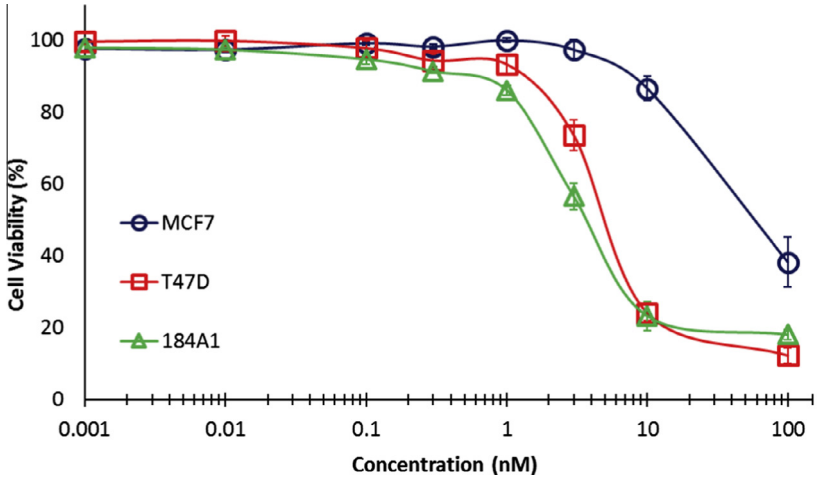


Figure 1. Percent cell viability of human breast cancer cell lines: MCF7, T47D, and 184A1 at various concentrations of **11**.

ymethyl)-*m*-carborane (**5a**) was afforded as a white solid with a yield of 59.3%. The di-substituted product (**5b**) (yield of 31.1%) and some starting material were also recovered and isolated using column chromatography on silica gel. The hydroxyl group was then protected using a trityl group so that the second carbon on the *m*-carborane cage could be manipulated. This was achieved using trityl chloride and Hünig's base to afford 1-(methyl triphenylmethyl ether)-1,7-dicarbadodecaborane (**6**) as a white solid with a yield of 92.3%.

Compound **6** was further modified by activating the unsubstituted carbon on the *m*-carborane moiety using *n*-butyllithium and then reacted with 1-chloro-4-iodobutane, producing 1-(methyl triphenylmethyl ether)-7-(4-chlorobutyl)-*m*-carborane (**7**) as a yellow oil with a yield of 100%. Following this, the trityl protecting group on compound **7** was removed using concentrated HCl to afford 1-(hydroxymethyl)-7-(4-chlorobutyl)-*m*-carborane (**8**) as a yellow oil with a yield of 87.2%. In a manner similar to that employed on **2**, the chlorine on compound **8** was exchanged with an azide using sodium azide to afford, 1-(hydroxymethyl)-7-(4-azidobutyl)-1,7-dicarbadodecaborane (**9**) as a yellow oil with a yield of 98.6%. Compound **9** was reduced using triphenylphosphine (PPh_3) instead of palladium on activated carbon. We observed the presence of secondary and tertiary amines in small quantities when reduction was done using palladium. Post reduction, 1-(hydroxymethyl)-7-(4-aminobutyl)-*m*-carborane (**10**) was afforded as an orange oil with 87.5% yield.

The hydroxymethyl derivative of MC4-PPEA (**11**) was synthesized using a method similar to that employed for **4** by converting *trans*-3-(3′-pyridyl)acrylic acid to *trans*-3-(3′-pyridyl)acryloyl chloride (PAC) in a one-pot, two-step reaction. To minimize a reaction between the hydroxyl group and PAC, both solutions of **10** and PAC were prepared separately and chilled to −78 °C using an acetone/dry-ice bath and the PAC solution was added drop-wise to the solution containing **10**. The reaction mixture was allowed to stir overnight, while gradually being allowed to warm to ambient temperature. This reaction afforded 1-(hydroxymethyl)-7-(4′-(*trans*-3″-(3‴-pyridyl)acrylamido)butyl)-*m*-carborane (**11**) as an orange foam with 75.9% yield.

The activity of **11** was evaluated using MTT assay,[26] against three human cell lines: MCF7, T47D, (cancerous) and 184A1 (a chemically transformed noncancerous breast epithelium). Figure 1 depicts the concentration dependent cell viability exhibited by **11**

against these cell lines in vitro. The compound exhibited half maximal inhibitory concentrations (IC_{50}) of 58.0 ± 5.2 nM, 12.0 ± 2.1 nM, and 3.9 ± 1.3 nM for MCF7, T47D, and 184A1 cell lines, respectively. Past studies, including our own, have demonstrated that the biological activity of Nampt inhibitors is cell line dependent and may vary over a decade, or more.[27] The present cell viability assays reveal that the addition of a hydroxymethyl moiety to **4**, producing compound **11**, results in an approximate 10-fold decrease in potency for the new molecule in these breast cancer cell lines. This new molecule exhibits low, to mid- nanomolar potencies in vitro and the presence of the hydroxymethyl group should allow for the covalent attachment of this molecule, through hydrolyzable linkers, to cancer-associated targeting vectors.

In conclusion, a significantly improved synthesis of MC4-PPEA has been described. A new hydroxymethyl functionalized derivative of **4** was also synthesized in high yield. Low, to mid-nanomolar potencies of the new derivative were observed in three human breast cell lines in vitro. The hydroxymethyl moiety of **11** should allow for the covalent attachment of this molecule, through hydrolyzable linkages, to cancer-associated targeting vectors, such as nanoparticles, proteins, or antibodies. The selective delivery of highly potent Nampt inhibitors to tumor tissue might overcome some of the limitations observed from early clinical trials of FK866/APO866.

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Supplementary data

Supplementary data (experimental procedures, characterization data for compounds, copies of NMR spectra, and information regarding cell culture and treatment) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.11.068.

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