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Preparation and Evaluation of Carborane Analogues of Tamoxifen

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A stereoselective synthesis of *closo* carborane analogues of tamoxifen was developed where the products represent a new approach to developing metabolically robust SERMs. The A-ring found in the backbone of tamoxifen was replaced with an ortho carborane cluster; the product was determined to be the desired *Z* isomer, which showed superior chemical stability to tamoxifen both in solution and in the solid state. By use of microwave heating, it was possible to convert some of the *Z* carborane tamoxifen analogue to the corresponding *E* isomer. Cell growth assays using both isomers and a carborane that is known to target the ER were conducted using estrogen receptor (ER) positive and ER negative human breast cancer cells with and without the presence of estradiol (E2). The *Z* carborane isomer was able to inhibit cell proliferation better than tamoxifen in an E2 free environment, while the *E* isomer inhibited cell growth better than tamoxifen when E2 was present.

Introduction

Tamoxifen (**1**, Figure 1) is currently used as a frontline treatment in cases of hormone dependent breast cancer. The agent is described by the World Health Organization (WHO) as an essential drug for the treatment of breast cancer.[1] In addition to acute cancer therapy, tamoxifen is used as a preventative treatment[2] for high risk woman as well as in long-term adjuvant therapy.[3] The use of tamoxifen however has also been associated with increased risk in developing endometrial[4,5] and uterine[6] cancers as well as increased possibility of suffering a pulmonary embolism, stroke, or deep vein thrombosis.[7] In light of this, several tamoxifen analogues (Figure 2) have been developed in hopes of retaining the benefits of the parent drug while reducing possible side effects.

Some of the early and more notable tamoxifen analogues include raloxifene (**2**),[8] idoxifene (**3**),[9] and the tamoxifen metabolite 4-hydroxytamoxifen (**4**).[10] Many of the analogues retain key structural elements of tamoxifen (**1**) that are critical in maintaining ER binding. This includes a pseudo-estradiol-like structure containing a hydrophobic core and several aromatic substituents around a central core.[11,12] There remains an active search for robust tamoxifen analogues that are more effective selective estrogen receptor modulators (SERMs[a]) particularly for patients who are likely to be using the agents for prolonged periods of time.

Endo et al. have reported several arylcarborane derivatives as estrogen analogues that have been shown to bind the estrogen receptor[13−16] (Figure 3) where the key structural components needed for binding are a phenol ring and a *closo* carborane unit. Carboranes are clusters of boron and carbon that have been used previously to prepare boron neutron capture therapy (BNCT) agents as a result of their high boron content.[17−20] What is less commonly known is that carboranes are interesting hydrophobic structural motifs for use in medicinal chemistry because of their unique 3D structures, ease of synthesis and functionalization, low toxicity, and resistance to catabolism in vivo.[21]

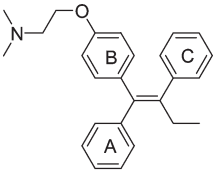

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Figure 1. Tamoxifen with aryl group labeling scheme.

To date, there have been no reports of a *closo* carborane analogue of tamoxifen. We previously reported the synthesis of a *nido* carborane analogue but were not able to prepare the complementary *closo* structures or to evaluate the *E* versus *Z* isomers.[23] Given the promising results of simple carborane phenol derivatives in binding to the ER, a synthetic method to *E* and *Z* carborane analogues of tamoxifen (Scheme 1) was developed and the products were tested in ER positive and negative cell lines.

Results and Discussion

Synthesis and Characterization. Carboranes are readily prepared from alkynes. Consequently a stereoselective method was developed to prepare the ene-yne **11** (Scheme 1). The preparation of the methoxy ketone **7** was adapted from Smyths and Corby[24] and was successfully scaled to produce multigram quantities (>100 g) where the product was combined with TMS-protected lithium acetylide. The resulting tertiary alcohol **8** was isolated as pale yellow oil and used

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[a] Abbreviations: ER, estrogen receptor; E2, estradiol; SERM, selective estrogen receptor modulator; TMS, trimethylsilyl; BNCT, boron neutron capture therapy; NOE, nuclear Overhauser effect.

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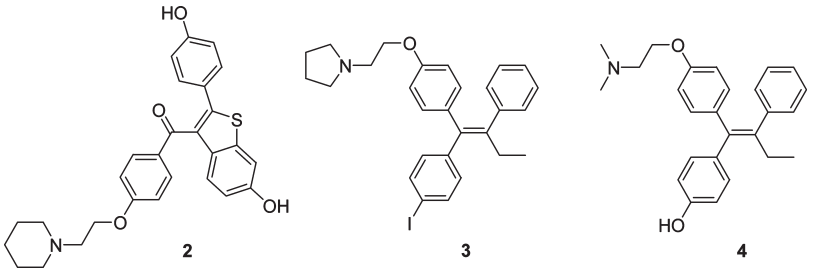


Figure 2. Tamoxifen analogues raloxifen (**2**), idoxifen (**3**), and 4-hydroxytamoxifen (**4**).

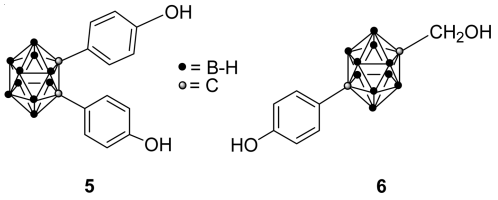


Figure 3. The *closo* carborane derivatives that bind the estrogen receptor.[22]

without further purification. It was immediately treated with thionyl chloride in pyridine at −78 °C and the corresponding protected ene-yne was isolated in 89% yield. The ^{1}H NMR of **9/10** showed a *Z*/*E* ratio of 15:1 which is consistent with the elimination reaction occurring via a concerted E2 elimination mechanism.

The TMS protecting group was removed by treating **9** and **10** with sodium methoxide in methanol (0.30 M). The reaction mixture was stirred for 24 h whereupon analysis of the mixture by thin layer chromatography (TLC) indicated complete consumption of the starting material. The light sensitive products **11** and **12** were isolated in 81% yield as a mixture via simple liquid−liquid extraction (dichloromethane/water). By use of ^{1}H nuclear Overhauser effect (NOE) spectroscopy, the major product was clearly identified as the *Z* isomer. Integration of the signals arising from the protons on the ethyl group (Figure 4) indicated that that the *Z*/*E* ratio was 93:7. The residual *E* isomer was later removed following isolation of the target carborane.

With significant quantities in hand of the acetylene derivative **11** having the appropriate stereochemistry, the alkyne insertion reaction was performed to generate the desired carborane. Initial attempts to obtain **13** utilizing a traditional alkyne insertion reaction failed to yield the target compound. This is likely the result of a combination of the highly conjugated nature of the ene-yne, which would reduce the overall activity of the compound to carborane formation, and the steric bulk around the reaction center. The ultimately successful approach involved the use of 1-butyl-3-methylimidazolium chloride (Bmim-Cl), an ionic liquid.[25] Decaborane and catalytic amounts of Bmim-Cl were dissolved in toluene to create a biphasic mixture to which **11** and **12** were added, and the mixture was heated at reflux for 12 h under argon. The removal of the reaction solvent yielded a dark solid which was purified via silica gel chromatography, and recrystallization gave the product as a white solid in 25% yield.

The ^{1}H NMR of **13** (Figure 5) was similar to that of the deprotected acetylene derivative with the exception that purification led to the isolation of a single *Z* isomer. This was evident looking at the triplet at 0.92 ppm (in Figure 5 for **13**) which lacks the corresponding signal from the *E* isomer (as seen at 1.06 (*Z*) and 0.92 ppm (*E*) in Figure 4). A broad peak was observed at 3.15 ppm that is attributable to the C−H of the carborane cage, while the B−H groups of the carborane form another broad signal spanning from 1 to 4 ppm.[26] The peaks in the ^{11}B NMR spectrum and the MS isotope pattern were consistent with the presence of a *closo* carborane cage, while the IR spectrum showed B−H stretches at 2605 cm^{-1}.

X-ray quality single crystals of **13** were obtained by dissolving the white solid in boiling petroleum ether, followed by slow evaporation over 9 days. The crystal structure of the *closo* carborane was consistent with the results of the NOE experiments and confirmed that the product was in fact the *Z*-isomer. The average C−C bond length of the carborane was 1.600(2) Å while the average B−B and C−B bond lengths were 1.782(3) and 1.719(2) Å, respectively, which are similar to the values reported for simple arylcarboranes.[23]

Removal of the methoxy protecting group in **13** was carried out using BBr_3 in ether to produce the phenol *closo* carborane **14** in 57% yield after recrystallization. The removal of the methoxy protecting group proved to be difficult and required a longer reaction time than those generally described in the literature.[27] This may be a result of the extended conjugation in **13** which would reduce the electron density on the methoxy oxygen, thereby slowing the rate of reaction. Despite the poor reactivity, the ^{1}H NMR spectrum of **14** clearly indicated the formation of the phenol through the loss of the signal associated with the methoxy group and appearance of signal associated with the phenol (δ 5.30 ppm). The IR spectrum further confirmed the formation of the phenol group (ν = 3573 cm^{-1}).

Single crystals of **14** were obtained by slow evaporation of a methanol solution, and the crystal structure (Figure 6) confirmed that **14** was the *Z*-isomer. The data obtained from the crystal structure allowed for comparison of the structural backbone of tamoxifen and the *closo* carborane analogue. From this information it was apparent that the introduction of the carborane had negligible effect on the structure of the tamoxifen backbone. The C1′−C9′ as well as the C1′−C2′ and C9′−C10′ bond lengths were found to be 1.348(2), 1.504(2), and 1.502(2) Å, respectively. The corresponding bond lengths in tamoxifen were found to be 1.347, 1.497, and 1.492 Å.[28]

When compounds **9**−**12** were dissolved in a solvent and exposed to light, they isomerized and degraded to form a mixture of compounds. In contrast, compound **14** could be stored for several months as a solid in an open container and as a methanol solution exposed to sunlight without any evidence of degradation. To further investigate the stability of **14**, a temperature course study was conducted (Table 1) by dissolving the compound in 95% ethanol and heating in a Biotage Initiator 60 microwave. The ^{1}H NMR of the reaction mixture showed that the compound did not degrade but in fact isomerized to form a mixture of *Z* and *E* isomers

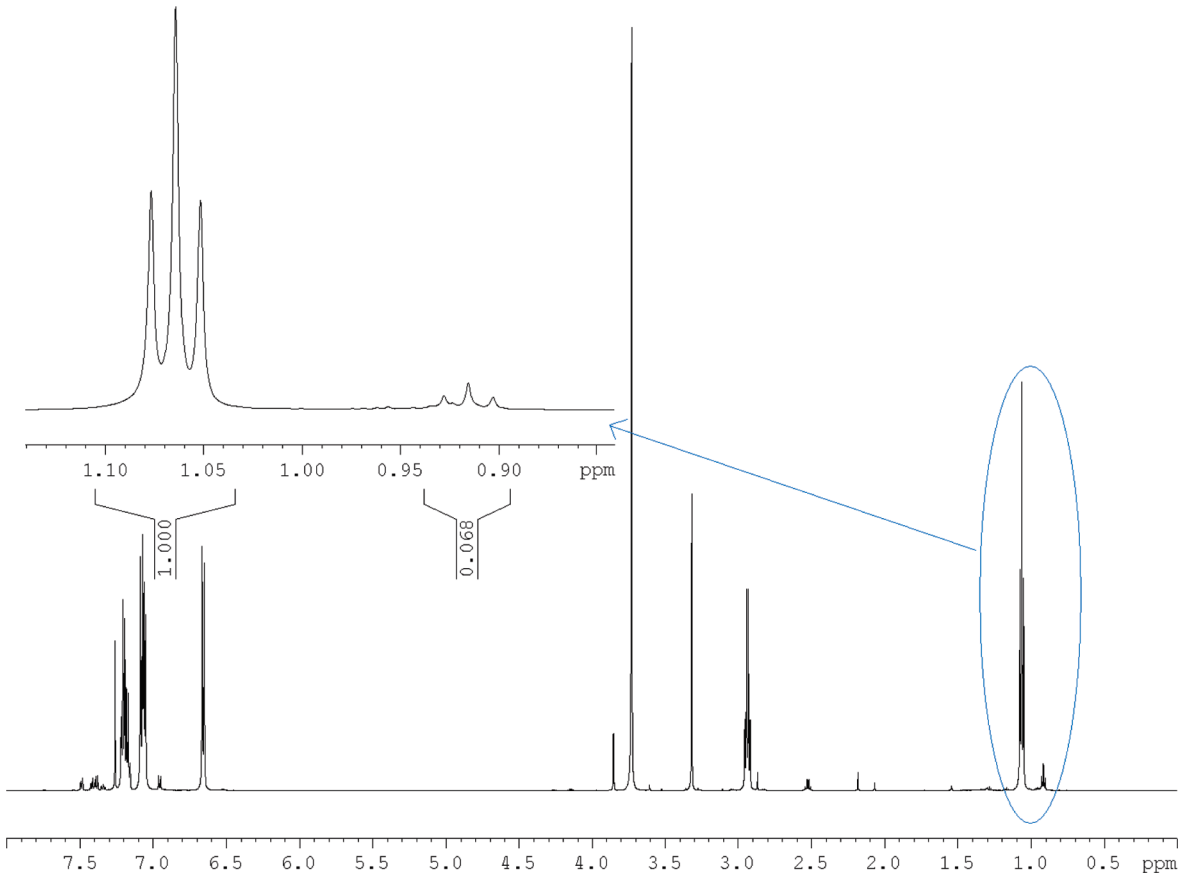
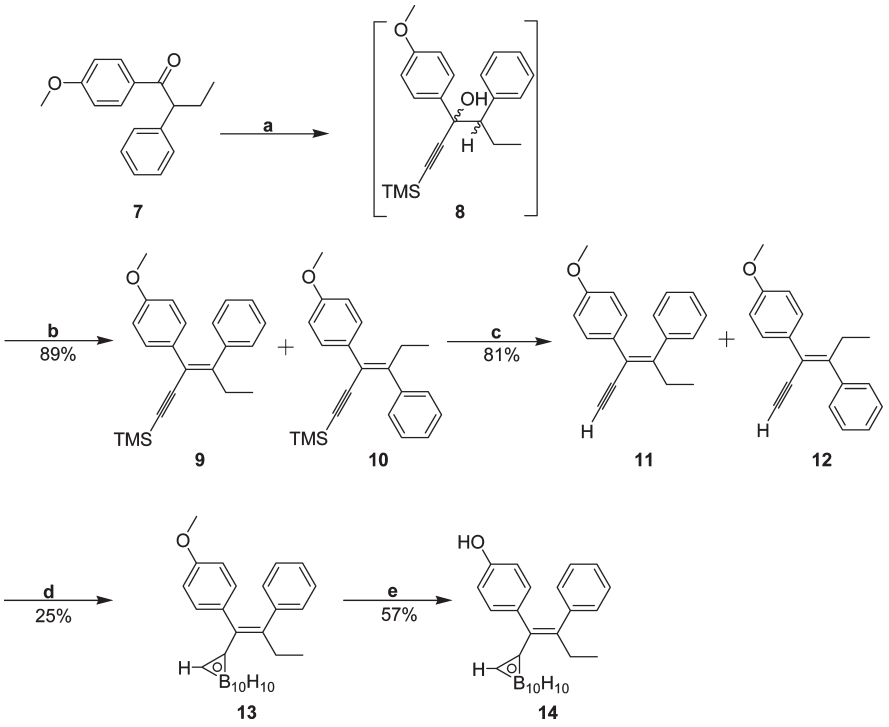


Figure 4. ^{1}H NMR of **11/12** following liquid−liquid extraction. Integration of the triplets in the highlighted region show a *Z*/*E* ratio of 15:1 (600 MHz, $CDCl_3$).

Scheme 1. Synthesis of **14**[a]



[a] (a) (1) TMS−acetylene, *n*-BuLi, −78°C, THF; (2) sat. ammonium chloride; (b) pyridine, thionyl chloride, −78 °C; (c) (1) NaOMe, MeOH; (2) H_2O; (d)1-butyl-3-methylimidazolium chloride, $B_{10}H_{14}$, toluene; (e) BBr_3, dichloromethane, −78 °C.

(Figure 7). The relative amount of each isomer present was determined by LC−MS.

In order to determine the identities of the two signals observed in the HPLC of **14** (Figure 8) following heating, the

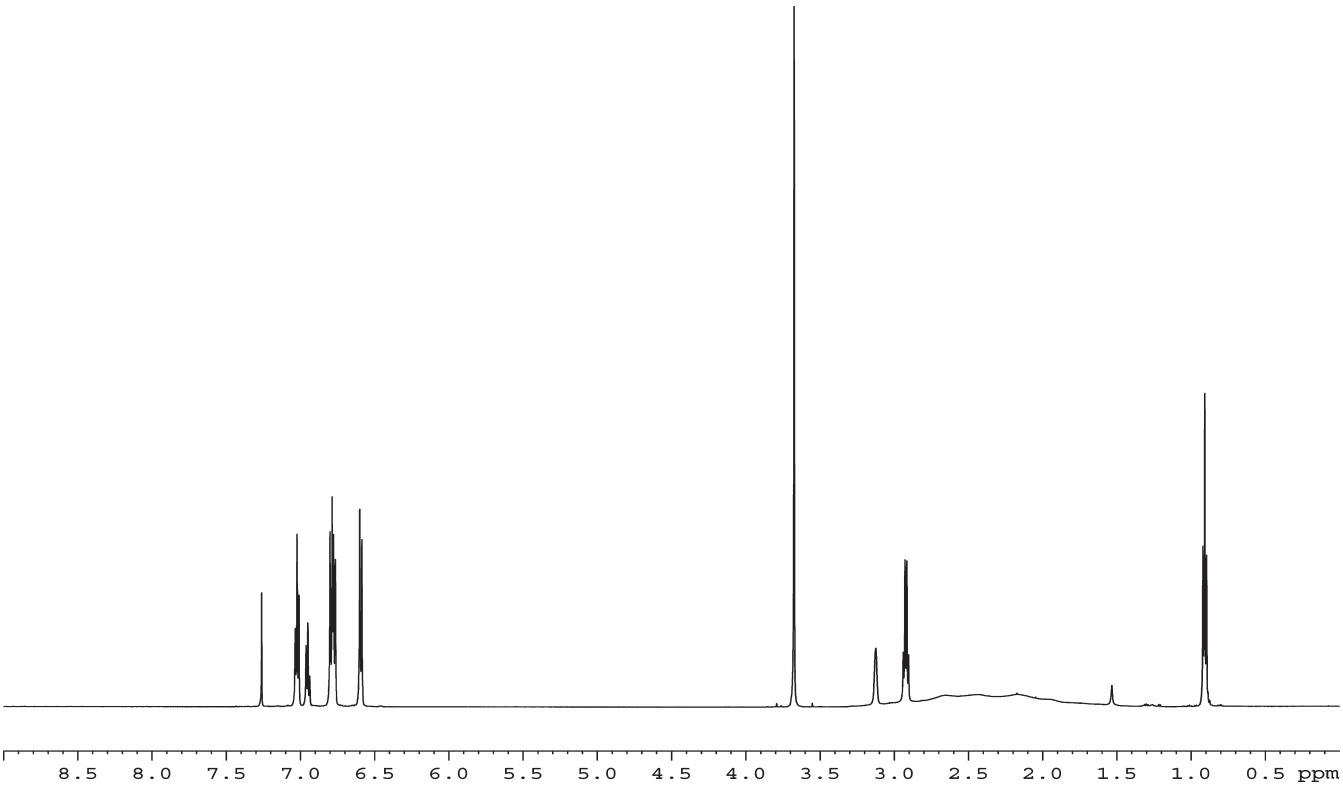


Figure 5. ^{1}H NMR of **13** (600 MHz, $CDCl_3$).

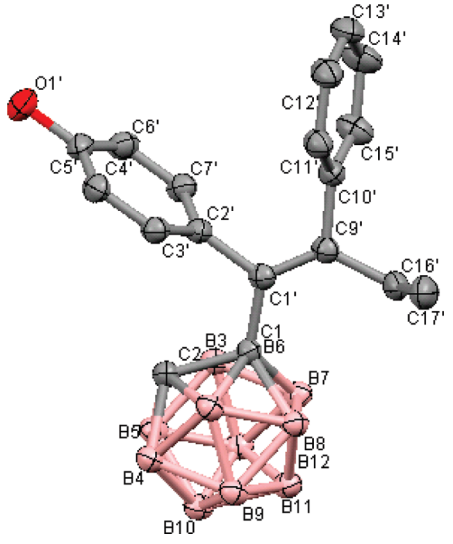


Figure 6. Crystal structure and atomic numbering scheme for **14** (50% thermal ellipsoids). Hydrogen atoms were omitted for clarity.

Table 1. *E*/*Z* Isomerization of **14** in 95% Ethanol Using Microwave Heating

temp (°C)	time (min)	*Z* (**14**) (%)	*E* (**15**) (%)
80	20	100	0
100	20	100	0
120	20	100	0
140	20	99	1
160	20	85	15
180	20	56	44

peak eluting at 20.8 min was isolated and the product fully characterized. The ^{1}H NMR spectrum showed that the product

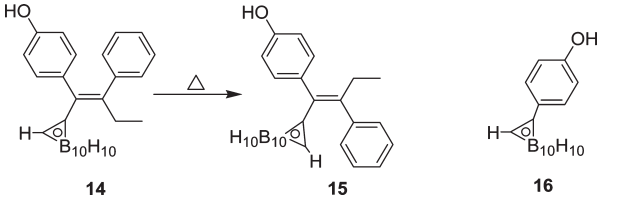


Figure 7. Thermal *E*−*Z* isomerization of **14** to give **15**. The structure of molecule **16** is shown.

was the *E*-isomer **15** as opposed to the starting *Z*-isomer (Figure 9). The most notable differences in the NMR were the upfield shift of the signals associated with the methylene group from 2.80 ppm in **14** to 1.89 ppm in **15** and the significant (0.5 ppm) downfield shift of the aromatic protons of **15** relative to those in **14**.

Biological Evaluation. Prior to screening, the purities of **14**, **15**, and 1-(4-hydroxyphenyl)-1,2-dicarba-*closo*-dodecaborane (**16**) were determined by reversed phase HPLC and found to be 98%, 97%, and 99%, respectively. Their ability to effect cell growth was evaluated using MCF-7 cells which are known to overexpress the estrogen receptor (ER)[29] as well as the MDA MB 231 cells that are known to have low endogenous ER expression.[27] Assays were done in comparison to **16**, a compound that has been previously prepared in our group[23] and by Endo et al.[30] which has shown the ability to bind to ER. Compounds were evaluated at five different concentrations (0.001, 0.01, 0.1, 1, and 10 μM) in the presence and absence of estradiol (E2) at four time points (1, 4, 7, and 10 days). Cell proliferation assays using MDA MB 231 cells showed no appreciable difference between the control samples and those incubated with **14**, **15**, or **16**.

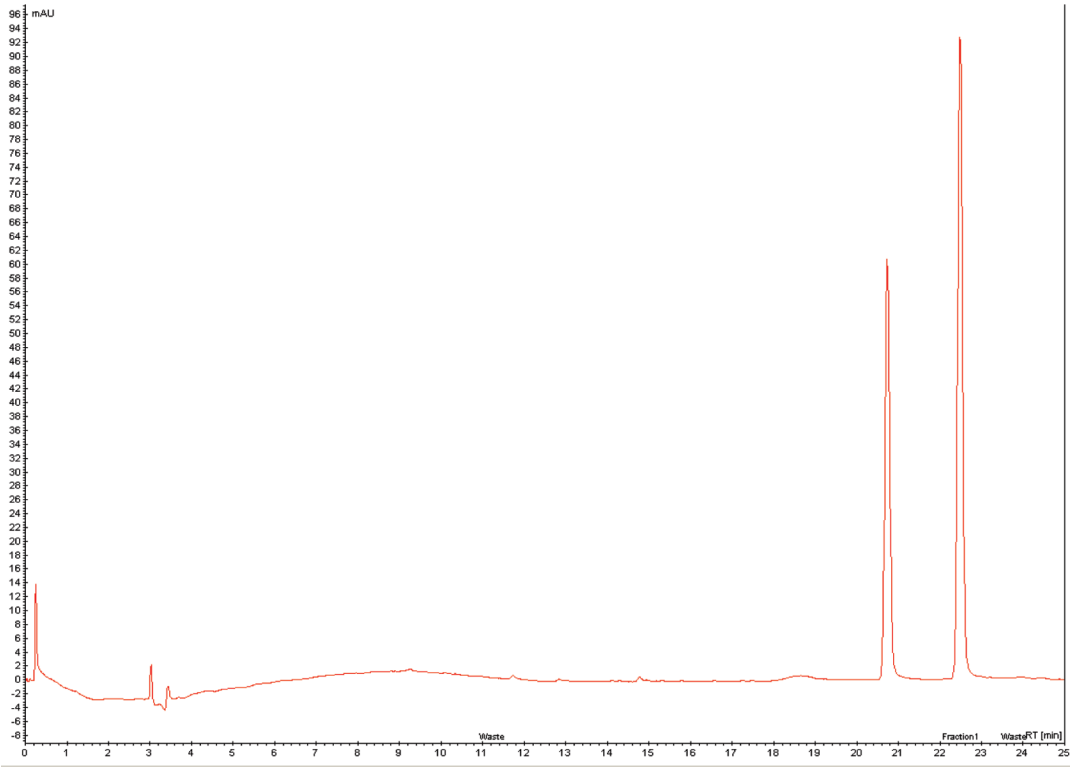


Figure 8. HPLC chromatogram of **14** following microwave heating at 180 °C for 20 min in 95% ethanol. Elution conditions were as follows. Solvent A = water, solvent B = acetonitrile. Gradient elution: 0−10 min, 80→20% A; 10−25 min, 20→0% A. Column: Zorbax RX-C18 (4.6 mm × 250 mm). Flow rate: 1 mL/min.

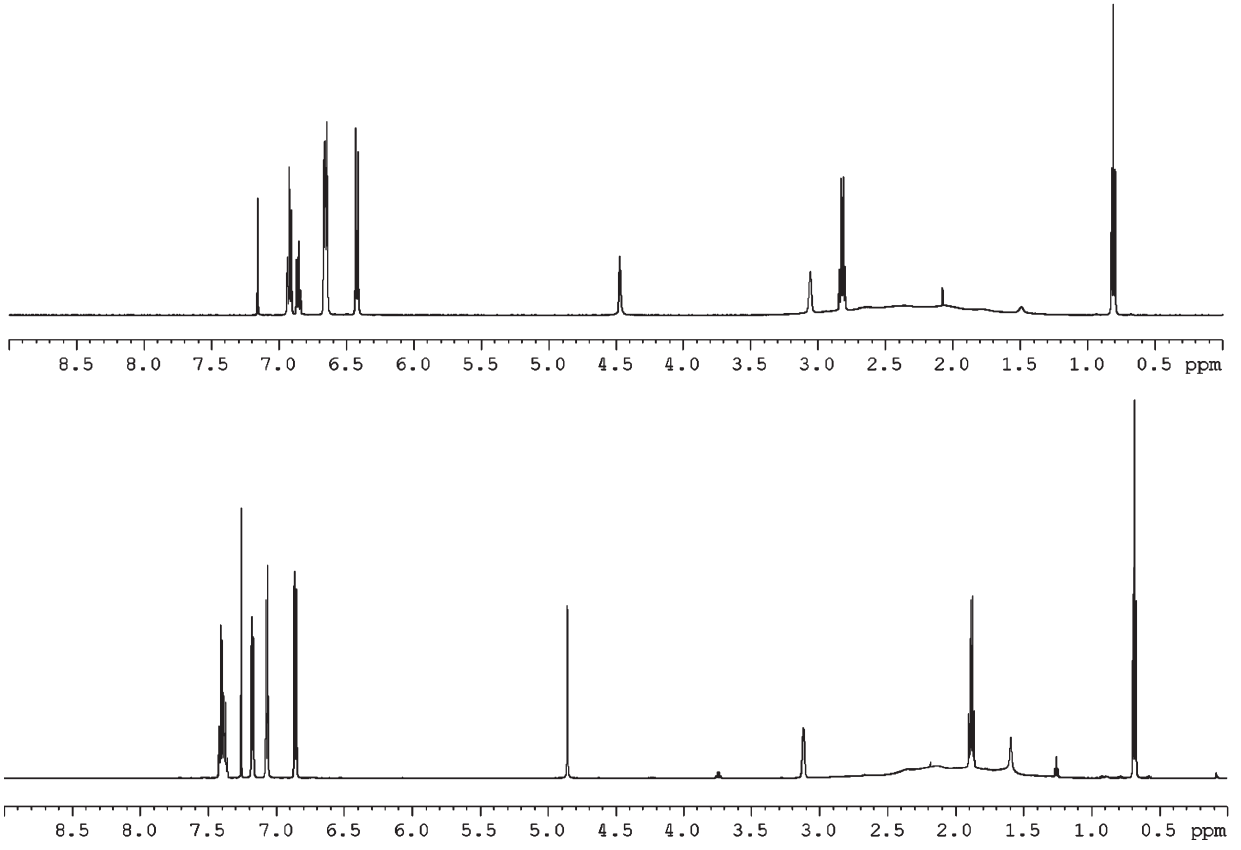


Figure 9. ^{1}H NMR spectra of **14** (top) and **15** (bottom) (600 MHz, $CDCl_3$).

In the MCF-7 cells, at 1 μM, **14** without E2 present (Figure 10A) showed nearly identical results compared to tamoxifen on all days, with day 10 generating a percent of control of 7.0 ± 0.2% and 5.8 ± 0.6%, respectively

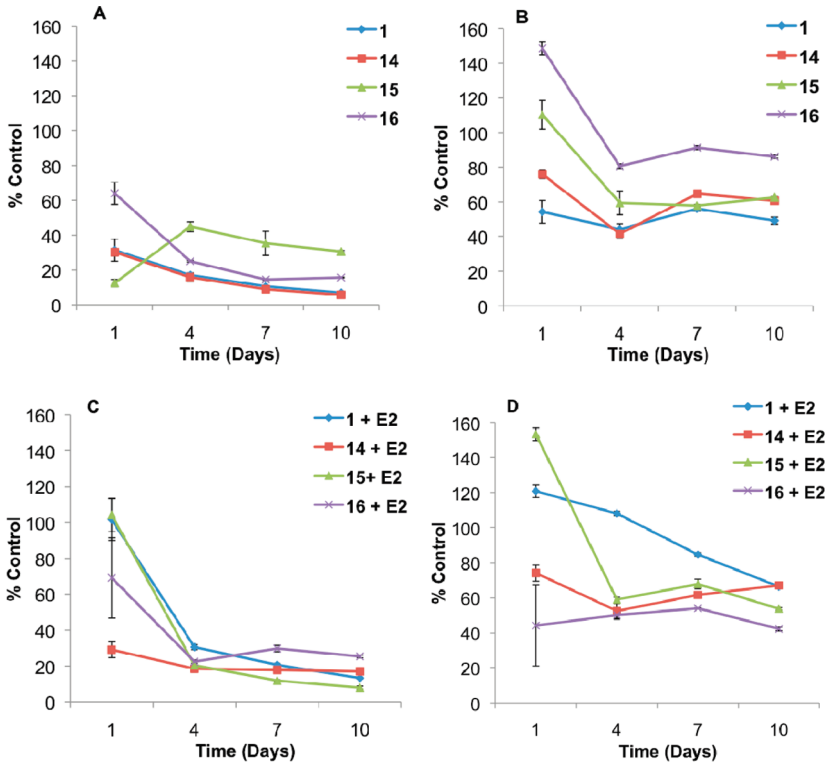


Figure 10. The results of cell proliferation assays reported a percentage of control for compounds **1**, **14**, **15**, and **16** at days 1, 4, 7, and 10: (A) 1 μM, (B) 0.001 μM, (C) 1 μM with E2, (D) 0.001 μM with E2. Parts A and B use growth data with no additives, and parts C and D use growth data plus 10 nM E2. Refer to Supporting Information for 0.01 and 0.1 μM graphs.

($p < 0.15$). At 0.001 μM, tamoxifen performed significantly better than **14**, showing on day 10 an average of 49.2 $\pm$ 2.10% of control cells compared to 60.7 $\pm$ 0.9% for **14** (Figure 10B). In the presence of 10 nM E2 (Figure 10C) the effect of 1.0 μM tamoxifen changed significantly over time with percent of control ranging from 101.7 $\pm$ 11.7% on day 1 to 13.3 $\pm$ 0.4% ($p < 0.006$) on day 10, which differs considerably from that for **14** (29.2 $\pm$ 4.4% to 17.1 $\pm$ 0.4%; $p < 0.00002$) under the same conditions. This result indicates that the onset of cell growth inhibition by **14** is more rapid than tamoxifen, which may indicate an inhibitory ER mechanism and/or a cytotoxic response. From Figure 10C it is evident that there is a slight difference in cell proliferation between tamoxifen (**1**) and **14** (13.3 $\pm$ 0.4 vs 17.1 $\pm$ 0.4, $p <$ 0.005) by day 10, which, although statistically different, is unlikely to be of biological significance. The plot also illustrates that **15** exhibits inhibitory action similar to tamoxifen, in that it is most effective at later time points, and its ability to inhibit cell growth was superior to **14** after day 4. The *E* tamoxifen analogue (**15**) exhibits superior inhibition of cellular proliferation compared to tamoxifen on days 4, 7, and 10, showing an average percent of control of 20.5 $\pm$ 2.6%, 11.9 $\pm$ 0.4%, and 8.0 $\pm$ 0.9% in comparison to 30.6 $\pm$ 1.4% ($p < 0.04$), 20.7 $\pm$ 0.8% ($p < 0.002$), and 13.3 $\pm$ 0.4% ($p < 0.02$), respectively. At 1 μM **15** with 10 nM E2, **15** shows greater inhibition of cell proliferation compared to **1**, **14**, and **16**; however, as the concentration of each drug was decreased with the level of E2 remaining constant, relative cell proliferation increased. At 0.001 μM compound +10 nM E2 (Figure 10D), at day 10, **16** showed the fewest number of viable cells (42.3 $\pm$ 1.0%, $p < 0.000\,07$) compared to tamoxifen (66.4 $\pm$ 0.9%), followed by **15** (53.8 $\pm$ 0.7%, $p < 0.0004$) and **14** (67.1 $\pm$ 1.2%, $p < 0.7$), which did not differ significantly from tamoxifen. In the presence of 10 nM E2, **14** and **16** show rapid reduction in cell numbers from day 1, which may indicate a cytotoxic response instead of solely an ER-based response. The proliferation values at 0.01 and 0.1 μM are between 1 and 0.001 μM, with similar patterns of cell growth inhibition over the 10-day incubation period. Graphs of cell proliferation for 0.01 and 0.1 μM concentrations can be found in Supporting Information.

Although there were significant differences between the ER-positive and -negative expressing cell lines in their response to the carborane compounds, it cannot be conclusively stated that ER-dependent pathways were the only mechanism of action of these analogues. It is well documented that tamoxifen has ER-independent mechanisms, which can reduce cell numbers, primarily through the induction of apoptotic pathways.[31−35] Now that it has been established that the carborane derivatives exhibit comparable or superior cytotoxic responses, a panel of receptor binding assays including ER binding measurements are currently underway to better elucidate the mechanism of action of the boron compounds.

Conclusion

The stereoselective synthesis of a *closo* carborane tamoxifen analogue was achieved in reasonable overall yield. It was determined that isomerization could be carried out using microwave heating to obtain a 50:50 mixture of the *E* and *Z* *closo* carborane isomers. Despite the isomerization, the *closo* carborane tamoxifen analogue has greater stability toward degradation than tamoxifen itself. The results of the cell proliferation assay indicate that the *Z* carborane tamoxifen analogue (**14**) shows similar inhibition compared to tamoxifen

in an E2 free environment, while in the presence of E2 the *E* carborane tamoxifen analogue (**15**) showed the lowest number of viable cells remaining after 4 days. These data in combination warrant further evaluation of the carborane analogues of tamoxifen.

Experimental Section

General. All reactions were carried out under argon that had been passed through Drierite, using commercial grade solvents dried using a Pure Solv MD-6 solvent purification system (Innovative Technology Inc.). Chemicals were purchased from Sigma-Aldrich or SGF chemicals and used without further purification. Decaborane was purchased from Katchem. Reactions requiring microwave heating were performed using a Biotage Initiator 60 instrument. Compound **7** was prepared following the literature procedure.[24] ^{1}H, ^{13}C, and ^{11}B NMR spectra were recorded on a Bruker AV600, Bruker DRX500, or Bruker AV200 spectrometer with probe temperatures of 30, 25, or 25 °C, respectively. ^{1}H NMR chemical shifts are reported in ppm relative to the residual proton signal of the NMR solvent. Coupling constants (J) are reported in hertz (Hz). ^{13}C chemical shifts are reported in ppm relative to the carbon signal of the solvent, while ^{11}B chemical shifts are reported in ppm relative to an external standard of $BF_3 \cdot Et_2O$. Thin layer chromatography plates (Merck F254 silica gel on aluminum plates) were visualized using 0.1% $PdCl_2$ in 3 M HCl(aq) and/or UV light. Infrared spectra were acquired using a BioRad FTS-40 FT-IR or Nicolet 510 FTIR spectrometer. High resolution mass spectra were obtained on a Waters/micromass Q-ToF Ultima Global spectrometer. Low resolution LCMS were obtained on a Waters 2695 LC with a Quattro Ultima triple quadrupole mass spectrometer. Analytical and semipreparative HPLC was performed using a Varian Pro Star model 330 PDA detector, model 410 autosampler, model 230 solvent delivery system, and model 710 fraction collector. Analysis was conducted using Agilent Zorbax SB-C18 (2a, 4.6 mm × 250 mm, and 2b, 9.8 mm × 250 mm) columns. Analytical HPLC experiments were monitored at 254 nm using a flow rate of 1.0 mL min^{-1} at 30 °C. Semipreparative HPLC experiments were monitored at 254 nm using a flow rate of 4.0 mL min^{-1} at room temperature. The elution protocol used was as follows. HPLC method A: solvent A = water, solvent B = acetonitrile. Gradient elution: 0−10 min, 80% → 20% A; 10−25 min, 20% → 0% A. Compounds were purified to at least 95% as determined by analytical HPLC.

Crystallographic Details. X-ray crystallographic data for **13** were collected from a single crystal frozen Paratone oil. Data were collected at 173 K on a Bruker Smart[36] Apex2Mo diffractometer using an Apex2 detector (512 × 512 mode) at 5 cm from the sample, three-circle D8 goniometer, 50 kV/30 mA fine focused sealed tube with graphite monochromator, and 0.50 mm pinhole collimator. Data were collected with ω and ϕ scans. Data for **14** was collected on a three-circle D8 Bruker diffractometer equipped with a Bruker SMART 6000 CCD area detector and a rotating anode utilizing cross-coupled parallel focusing mirrors to provide monochromated Cu Kα radiation (λ = 1.541 78 Å). Data processing for both compounds was carried out by use of the program SAINT,[37] while the program SADABS[38] was utilized for the scaling of diffraction data, the application of a decay correction, and an empirical absorption correction based on redundant reflections. The structures were solved by using the direct-methods procedure in the Bruker SHELXTL program library[39] and refined by full-matrix least-squares methods on F^2. All non-hydrogen atoms were refined using anisotropic thermal parameters, and hydrogen atoms were located using the difference map and refined using isotropic thermal parameters. CCDC 781744 and 781745 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Syntheses. (*Z*/*E*)-(3-(4-Methoxyphenyl)-4-phenylhex-3-en-1-ynyl)trimethylsilane (9/10). Trimethylsilylacetylene (35 mL, 250 mmol) was added to dry THF (750 mL) that had been chilled to −78 °C. To this, *n*-BuLi (100 mL, 0.25 mol) was added dropwise to form a clear colorless solution. Compound **7** (63.5 g, 250 mmol) dissolved in dry THF (500 mL) was added over 30 min whereupon the mixture turned bright pink. The reaction mixture was allowed to warm to room temperature over 12 h. To the pale yellow solution chilled to 0 °C, saturated ammonium chloride solution (400 mL) was added followed by water (800 mL). The mixture was separated into two fractions, and each was extracted with diethyl ether (3 × 500 mL) which was pooled and dried over anhydrous sodium sulfate and evaporated giving a yellow oil. The oil (**6**) was dissolved in 40 mL of anhydrous pyridine and THF (500 mL). The solution was cooled to −78 °C, and thionyl chloride (16 mL, 185 mmol) was added dropwise over 30 min. The reaction mixture was allowed to warm to room temperature over 12 h. Water (200 mL) was added to the pale yellow solution, followed by 2 M HCl (100 mL). The organic and aqueous layers were separated, and the organic fraction was washed with 2.0 M HCl (2 × 600 mL). The aqueous fractions were washed with diethyl ether (3 × 500 mL) and all organic fractions combined, dried over anhydrous sodium sulfate, and evaporated to give a brown oil (75 g, 89%). TLC (7.5:1 hexanes/ethyl acetate) R_f=0.78. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.25−6.60 (m, 9H, aryl), 3.75 (s, 3H, -O-CH_3), 2.90 (q, J = 7.5 Hz, 2H, CH_3), 1.05 (t, J = 7.5 Hz, 3H, CH_2), 0.25 (s, 9H, -S-$(CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 158.1, 151.8, 140.8, 131.0, 129.0, 128.0, 126.8, 119.4, 113.0, 105.9, 98.3, 55.1, 31.4, 0.1. m/z calculated for $[M + H]^+$ $C_{22}H_{27}OSi$: 335.1831. HRMS EI+ $[M + H]^+$ 335.1847. FTIR (NaCl, cm^{-1}) ν: 1608, 2138, 2962.

(*Z*/*E*)-1-Methoxy-4-(4-phenylhex-3-en-1-yn-3-yl)benzene (11/12). Compounds **9** and **10** (75 g, 224.5 mmol) were dissolved in a solution of sodium methoxide (16 g, 305.5 mmol) in methanol (1 L). The mixture was stirred for 24 h at room temperature. Water (500 mL) was added, and the mixture was extracted with dichloromethane (3 × 600 mL), which in turn was extracted with water (3 × 600 mL). The organic layer was evaporated to give a yellow oil. Yield (53 g, 81%). TLC (7.5:1 hexanes/ethyl acetate): R_f = 0.46. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.21−6.65 (m, 9H, aryl), 3.72 (s, 3H, OCH_3), 3.31 (s, 1H, CH), 2.94 (q, J = 7.5 Hz, 2H, CH_2), 1.06 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ158.3, 152.4, 140.6, 131.0, 130.0, 128.6, 128.1, 118.5, 113.2, 84.5, 81.4, 55.2, 31.3, 12.6. m/z calculated for $[M + H]^+$ $C_{19}H_{19}O$: 263.1436. HRMS $[M + H]^+$ EI+ 263.1430. FTIR (NaCl, cm^{-1}) ν: 1608, 2088, 2969, 3288.

(*Z*)-1-(1,2-Dicarba-*closo*-dodecaborane-1-yl)-1-(4-methoxyphenyl)-2-phenylbut-1-ene (13). 1-Butyl-3-methylimidazolium chloride (2.8 g, 89.0 mmol) and decaborane (12 g, 99 mmol) were dissolved in dry toluene (500 mL), forming a biphasic mixture. The solution containing a mixture of **11** and **12** (22.0 g, 84.0 mmol) dissolved in dry toluene was added dropwise and the reaction mixture heated to reflux for 12 h. The mixture was filtered and then subsequently evaporated to yield a dark solid which was purified by column chromatography (100% hexanes → 7.5:1 hexane/ethyl acetate) to yield a pale yellow liquid, which upon cooling yielded a white solid. The white solid was recrystallized from low boiling petroleum ether to yield white crystals (8.0 g, 25%). Mp: 120−121 °C. TLC (7.5:1 hexanes/ethyl acetate): R_f = 0.55. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.2−6.6 (m, 9H, aryl), 3.67 (s, 3H, -O-CH_3), 3.15 (s, 1H, $C_{carborane}$-H), 2.95 (q, J = 7.0 Hz, 2H, CH_2), 0.92 (t, J = 7.0 Hz, 3H, CH_3). ^{11}B{^{1}H} NMR (160 MHz, $CDCl_3$): δ −3.7, −8.9, −10.1, −12.3, −14.2. ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.7, 151.2, 142.7, 131.8, 131.4, 129.4, 129.3, 128.5, 127.5, 126.1, 113.7, 63.2, 55.2, 27.7, 12.8. m/z calculated for

$C_{19}H_{28}B_{10}O$: [M + formate]$^-$ and [M + acetate]$^-$ 425.3129 and 439.3286. HRMS TOF EI$^-$ [M + formate]$^-$ and [M + acetate]$^-$ 425.3120 and 439.3281. FTIR (NaCl, cm^{-1}): ν 1605, 2605, 3071.

(*Z*)-1-(1,2-Dicarba-*closo*-dodecaborane-1-yl)-1-(4-phenol)-2-phenylbut-1-ene (14). Compound **13** (1.0 g, 2.63 mmol) was dissolved in dry dichloromethane and cooled to −78 °C. BBr_3 (4.0 mL, 4.0 mmol) was added over 5 min, and the mixture was allowed to warm to room temperature over 12 h and stirred for an additional 36 h. The mixture was evaporated to dryness and the residue redissolved in methanol and evaporated. The brown solid was recrystallized from low boiling petroleum ether, yielding **13** as a colorless solid (0.5 g, 57%). Mp: 179−181 °C. TLC (7.5:1 hexanes/ethyl acetate): R_f = 0.27. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.9 −6.4 (m, 9H, aryl), 4.4 (s, 1H, -OH), 3.05 (s, 1H, $C_{carborane}$-H), 2.80 (q, J = 7.5 Hz, 2H, CH_2), 0.80 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.2, 150.5, 141.9, 131.2, 130.7, 128.2, 127.7, 126.8, 125.4, 114.5, 67.4, 62.4, 26.9, 25.0, 12.0. ^{11}B{^{1}H} NMR (160 MHz, C_3D_6O): δ −3.1, −8.4, −9.2, −11.1, −13.2. m/z calculated for [M − H]$^-$ $C_{18}H_{25}B_{10}O^-$: 365.2918. HRMS TOF EI$^-$ [M − H]$^-$ 365.2910. FTIR (NaCl, cm^{-1}) ν: 1609, 2608, 3573.

(*E*)-1-(1,2-Dicarba-*closo*-dodecaborane-1-yl)-1-(4-phenol)-2-phenylbut-1-ene (15). Compound **14** (72 mg, 0.196 mmol) was dissolved in 95% ethanol (2.0 mL) and heated using microwave irradiation at 185 °C for 25 min. An aliquot of the reaction mixture (24 mg) was purified via semipreparative HPLC (method A, column 2b), yielding a white solid (8.4 mg, 35%). Mp: 144−145 °C. TLC (7.5:1 hexanes/ethyl acetate): R_f = 0.34. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41 −6.85 (m, 9H, aryl), 4.86 (s, 1H, OH), 3.12 (s, 1H, carborane CH), 1.89 (q, J = 7.5 Hz, 2H, CH_2), 0.69 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 155.2, 147.7, 138.8, 132.1, 131.5, 129.7, 128.4, 128.0, 127.7, 115.2, 59.1, 33.7, 11.7. ^{11}B{^{1}H} NMR (192 MHz, $CDCl_3$): −3.5, −10.2, −11.1, −13.9. m/z calculated for [M − H]$^-$ $C_{18}H_{25}B_{10}O^-$ 365.2918. HRMS TOF EI$^-$ [M − H]$^-$ 365.2920.

Cell Lines and Tissue Culture. MCF-7 and MDA MB 231 human breast adenocarcinoma cells lines were obtained from ATCC (Manassas, VA). Both cell lines were cultured in DMEM without phenol red (CA12001-630, VWR International, Mississauga, Ontario, Canada) supplemented with 10% charcoal-stripped fetal bovine serum (CA95039-622, VWR International), 1% L-glutamine (25030-081, Invitrogen, Mississauga, Ontario, Canada), and 1% antibiotic/antimycotic (AB/AM) (15240-062, Invitrogen). All cells were maintained at 37 °C in 5% CO_2.

Growth Inhibition Study. MCF-7 and MDA MB 231 cell lines were used to assess whether **14**, **15**, and **16** were effective inhibitors of cell proliferation in ER positive and ER negative expressing cells. Both cell lines were plated (5.0×10^4 cells) in each well of six-well plates (9.51 cm^2 growth surface) in 2 mL of medium 24 h prior to the start of the experiment to allow for cell adhesion to growth surface and repopulation of cell surface receptors. Each cell line had 46 treatment groups (three replicate wells per group) which included control (no treatment), 0.001, 0.01, 0.1, 1, and 10 μM estradiol, tamoxifen, **14**, **15** or **16** alone, or compound plus 10 nM estradiol (excluding estradiol group). At the start of the experiment (day 0), the media were removed from each culture and replaced with 2 mL of supplemented media containing the appropriate concentration of the experimental compound. The compound levels were maintained by replacing the culture media with fresh compound containing media every 3 days.

Triplicate wells of each treatment group were harvested on days 1, 4, 7, and 10 to determine total cell number/well. Media were removed from each well and discarded, and the cells were gently rinsed with 1 mL phosphate buffered solution (PBS). Cells were released from the growth surface by the addition of 500 μL of 0.05% trypsin/EDTA to each well, followed by the addition of 1 mL of media to inactivate the trypsin. The growth surface was flushed 3 times to ensure the removal of all cells. An aliquot (50 μL) of the resulting cell suspension was placed in a microcentrifuge tube for counting. An equivalent volume of the viability dye Trypan Blue was added to the cell suspension and mixed well, and 10 μL of the resulting solution was pipetted onto a hemocytometer. The cells within four grids were counted and averaged to obtain a count of the total cells in each well. A Leitz Dialux light microscope was used for the analysis.

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Supporting Information Available: Two crystallographic files in CIF format; NMR, IR, and MS spectral data for all novel compounds; and X-ray data for compounds **13** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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