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Synthesis, stereochemistry confirmation and biological activity evaluation of a constituent from *Isodon excisus*

Xuechao Xing, Pei Ho, Geoffroy Bourquin, Li-An Yeh and Gregory D. Cuny*

Laboratory for Drug Discovery in Neurodegeneration, Brigham and Women's Hospital and Harvard Medical School, 65 Landsdowne St., Cambridge, MA 02139, USA

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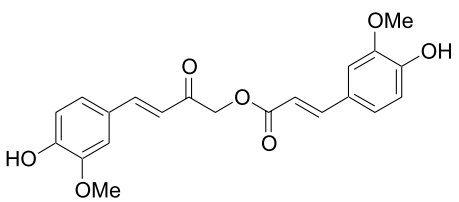
Abstract—A synthesis and stereochemistry confirmation of a constituent recently isolated from the whole plant *Isodon excisus* is reported. An enantioselective catalytic boron-mediated reduction of an α-bromoketone was utilized in the key synthetic transformation. The methodology described herein was also used for the synthesis of the natural product's enantiomer and several derivatives. In addition, the compounds were evaluated for inhibitory activity in a caspase induction assay. The natural product was found to be devoid of activity, but several derivatives had moderate inhibitory activity ($EC_{50}<1$ μM).

1. Introduction

Neurodegenerative diseases are a group of maladies that afflict a significant portion of the human population. The medical and socio-economic impacts of these diseases are significant. Although the etiology of each neurodegenerative disease is likely different, one common feature that many share is progressive and irreversible neuronal cell death in specific regions of the central nervous system.[1] Compelling evidence is emerging that one of the primary mechanisms responsible for the observed neuron cell death in Parkinson's disease (PD),[2] Huntington's disease (HD),[3] amyotrophic lateral sclerosis (ALS)[4] and human immunodeficiency virus associated dementia (HAD)[5] is apoptosis. Studies have also suggested that this mode of cell death may also be involved in Alzheimer's disease (AD).[6] Albeit, neurons present in AD may be chronically dysfunctional without necessarily undergoing active cell death.[7] Apoptosis is a genetically regulated process involving an elaborate sequence of biochemical events culminating in cell death and disassembly.[8] Many key cellular components in this cascade have been identified and some serve as potential targets for therapeutic intervention including caspases, which are a family of cysteine proteases.[6,9] Identifying and preparing low molecular weight molecules that interact directly with or alter the expression of these cellular constituents can assist in the understanding of the pathophysiology of disease and can provide lead compounds for therapeutic development.

Recently, two structurally related natural products, calebin A (**1**),[10a] isolated from turmeric (*Curcuma longa* L., Zingiberaceae) and **2a**,[11] isolated from the whole plant *Isodon excisus*, have been independently reported. Both compounds were described as apoptosis inhibitors. Compound **1** protected PC12 rat pheochromocytoma and IMR-32 human neuroblastoma cells from β-amyloid insult,[10a] while **2a** has been reported to inhibit etoposide-induced apoptosis in U937 cells by impeding the induction of caspase-3/7.[11] A synthesis of calebin A (**1**) and several analogs has recently been published.[10b]

In the present study, we report the synthesis of **2a**, its enantiomer **2b** and several derivatives. The synthesis described also allowed for a confirmation of the absolute stereochemistry of the natural product reported by Kho et al.[11] They inferred the stereochemistry based on the work reported by Zwanenburg, et al.[12a] and Huang et al.[12b] In addition, the compounds were evaluated for their ability to inhibit etoposide-induced caspase induction in U937 cells.



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Keywords: apoptosis; caspase-induction; inhibitor; boron; *Isodon excisus*.
* Corresponding author. Tel.: +1-617-768-8640; fax: +1-617-768-8606; e-mail: gcuny@rics.bwh.harvard.edu

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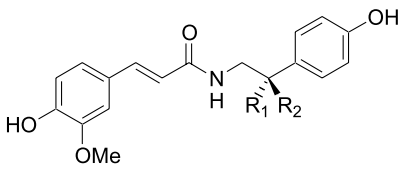
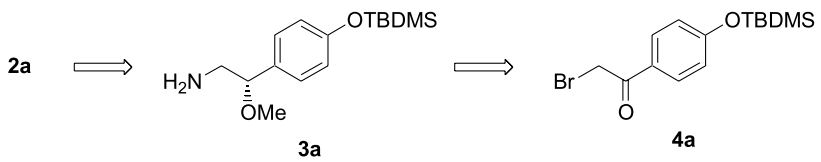


2a: R_1=OMe; R_2=H
2b: R_1=H; R_2=OMe

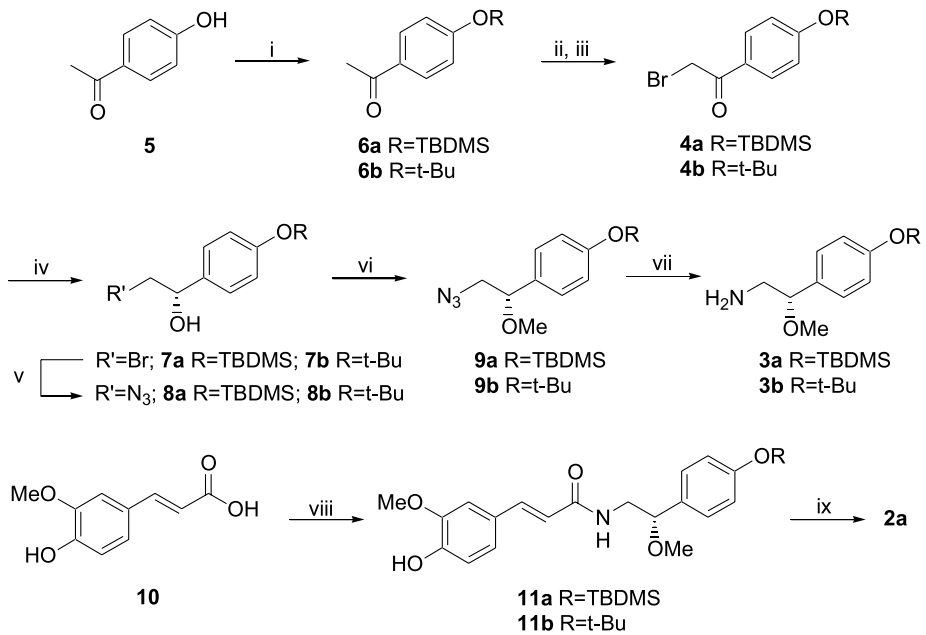
2. Results and discussion

A retrosynthetic analysis of **2a** began with a disconnection of the amide bond to give **3a**. Lawrence and Bushell reported an enantioselective synthesis of a structurally similar amine utilizing an asymmetric dihydroxylation of a styrene derivative in the synthesis of chelonin B,[13a] while Hergenrother recently described the regio- and chemoselective synthesis of 1-aryl-2-amino ethanol utilizing asymmetric aminohydroxylation of styrenes.[13b] However, we envisioned the assembly of the aminoether **3a** via an enantioselective catalytic boron-mediated reduction of α-bromoketone **4a** as the key synthetic transformation followed by introduction of the amine and alkylation of the alcohol in a manner similar to Zaponakis and Katerinopoulos (Scheme 1).[13c]

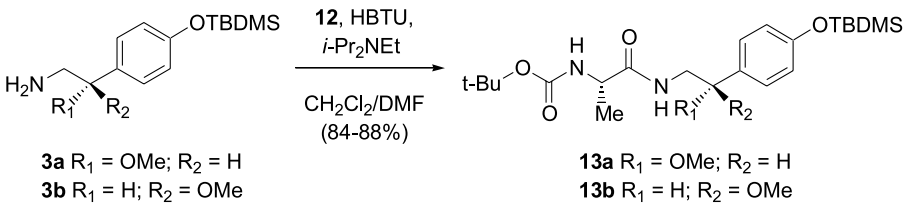
The synthesis began by preparing the *tert*-butyldimethylsilyl (TBDMS) ether **6a** from 4-hydroxyacetophenone (**5**, Scheme 2).[14] Next, the α-bromoketone **4a** was generated through a two step process. First, **6a** was allowed to react with trimethylsilyl triflate (TMSOTf) in the presence of diisopropylethylamine (*i*-Pr_2NEt) to generate the corresponding silyl enol ether in situ. Then bromination was effected with *N*-bromosuccinimide (NBS) to yield **4a** in 97% yield.[15] The α-bromoketone **4a** was reduced enantioselectively with borane–methyl sulfide complex and a catalytic amount of (*S*)-2-methyl-CBS-oxazaborolidine utilizing methodology developed by Corey et al. to give the bromohydrin **7a** in 89% yield.[16–18] Displacement of the bromine (0.27 M in water) with sodium azide employing phase-transfer catalysis with tetrabutylammonium bromide (TBABr) provided **8a** in moderate (60%) yield.[19] Next, alcohol **8a** was converted to ether **9a** in 50–90% yield via deprotonation with sodium hydride followed by alkylation with methyl iodide.[20a] Alternatively, alcohol **8a** was alkylated with methyl iodide in the presence of silver oxide to give ether **9a** in 72% yield.[20b] Reduction of the azide was readily accomplished by hydrogenation (H_2, 1 atm) in the presence of 5% Pd/C to give **3a**.[21] The unpurified amine **3a** was coupled utilizing *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (HBTU)[22] in dichloromethane to the cinnamic acid



Scheme 1.



Scheme 2. (i) *t*-$BuMe_2SiCl$, imidazole, DMF, 0°C to room temperature, 98%; (ii) TMSOTf, *i*-Pr_2EtN, DCM, 0°C, 30 min; (iii) NBS, 0°C, 4 h, 97% for two steps; (iv) $BH_3{\cdot}Me_2S$, (*S*)-2-methyl-CBS-oxazaborolidine, 0°C to room temperature, 89%; (v) NaN_3, TBABr, H_2O, 40°C, 60–80%; (vi) NaH in THF then MeI, 50–90% or Ag_2O, MeI, 70°C, 72%; (vii) H_2 (1 atm), 5% Pd/C, MeOH; (viii) **3a** or **3b**, HBTU, *i*-Pr_2EtN, DCM, 86% for two steps; (ix) TBAF, THF, 97%.



Scheme 3.

derivative **10**, which was prepared from the corresponding commercially available ethyl ester[23] to yield **11a** (86% for two steps). HBTU was not very soluble in dichloromethane. However, addition of small amounts of dimethylformamide to increase its solubility was detrimental, leading to formation of undesired side products. Finally, deprotection of the silyl ether was accomplished using tetrabutylammonium fluoride (TBAF) to give the natural product **2a**. The ^{1}H NMR spectra of the synthetic product was identical to that reported for the natural product.

The enantiomer **2b** was also prepared employing the same synthetic protocols described for **2a**, except that (*R*)-2-methyl-CBS-oxazaborolidine was utilized in the enantioselective boron-mediated reduction of α-bromoketone **4a**. Furthermore, starting with 4-*tert*-butoxyacetophenone (**6b**), the *tert*-butoxy derivative **11b** was also prepared utilizing the same methodology. The synthesis proceeded in a similar manner, except for the conversion of bromide **7b** to azide **8b**, which occurred in higher yield (88%).

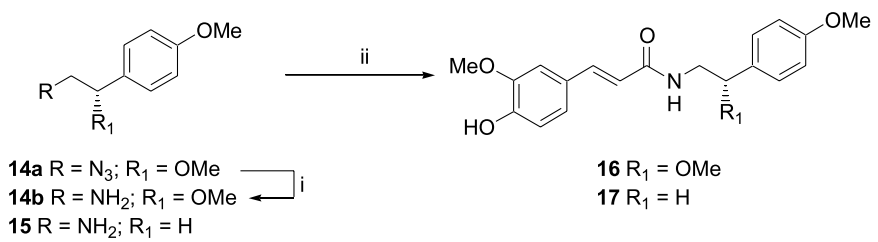
The enantiomeric purity of **3a** was assessed by analysis of the corresponding L-alanine derivative **13a** (Scheme 3). This material was prepared by allowing **3a** to react with *N*-Boc-L-alanine (**12**), in the presence of HBTU and *i*-Pr$_2$NEt in dichloromethane/DMF (10:1). Likewise, diastereomer **13b** was prepared from **3b** in a similar manner.[24] Analysis of the ^{1}H NMR spectra of **13a** and **13b** clearly demonstrated very high diastereomeric purities for each, indicative of high enantiomeric purities (>98% ee) for **3a** and **3b**, respectively. Racemization of the chiral center during the remaining reactions in the synthesis of **2a** and **2b** are quite improbable.

Two additional compounds were prepared for the structure–activity relationship (SAR) study (Scheme 4). Azide **14a** was prepared from **8a** utilizing sodium hydride, methyl iodide and prolonged reaction time. Presumably under these conditions the *tert*-butyldimethylsilyl group in **8a** was cleaved to give a phenol that was subsequently alkylated along with the aliphatic alcohol upon addition of methyl iodide. The product was then hydrogenated to give amine **14b**. Coupling of amines **14b** (unpurified) and **15** with the cinnamic acid derivative **10** utilizing HBTU provided amides **16** and **17**, respectively.

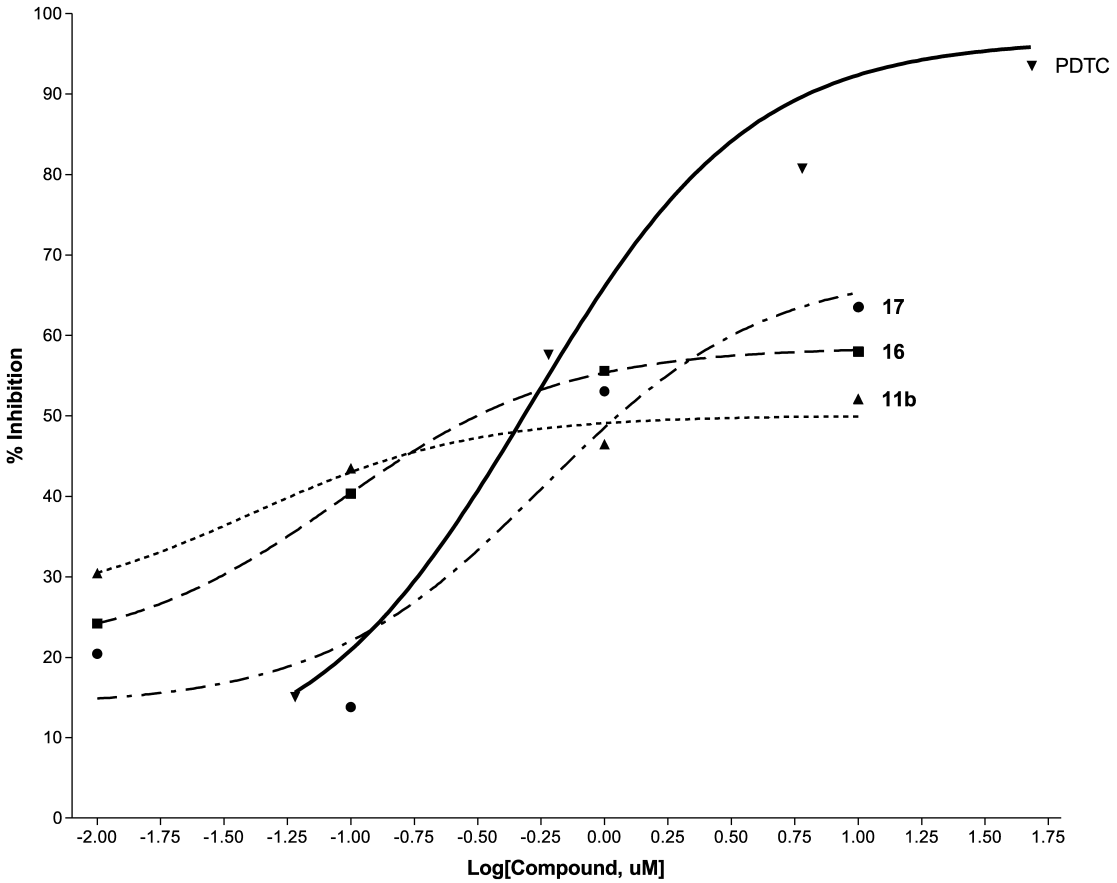
Compounds **2a** and **2b** were evaluated for their ability to inhibit etoposide-induced caspase induction in U937 cells. Etoposide (40 μM) was added to the cells (4×10^4 cells/well) in the presence or absence of various concentrations of **2a**, **2b** or ammonium pyrrolidinedithiocarbamate (PDTC). Following incubation (4.5 h), caspase-3/7 protease activity was determined using an Apo-ONE Homogeneous Caspase-3/7 Assay kit that employs z-DEVD-R110 as substrate. This modified peptide substrate produces a fluorescent rhodamine product upon enzymatic cleavage. The incubation time of 4.5 h was selected because it provided robust induction of caspase-3/7 with cells undergoing apoptotic cell death as observed microscopically in agreement with literature reports.[25] The positive control, PDTC, was able to prevent caspase induction with an EC_{50}=0.51 μM (Graph 1). However, neither the natural product **2a** nor its enantiomer **2b** were active even at high concentration (>10 μM). Therefore, we have been unable to confirm the caspase induction inhibitory activity previously attributed to this natural product.[11,26] Similar results were obtained with various concentrations of etoposide (10 and 20 μM) and with different cell numbers (1×10^4 and 2×10^4 cells/well).

The derivatives **11b**, **16** and **17** were similarly evaluated (Graph 1). The *tert*-butoxy derivative **11b** was found to have activity (0.1–10 μM) with a maximum inhibition of 50%. Derivative **16** was quite potent (EC_{50}=100 nM) at inhibiting caspase induction, but only provided a maximum inhibition of ~60%. However, derivative **17**, which lacks the aliphatic methoxy group, was moderately potent (EC_{50}=570 nM) and provided a maximum inhibition of ~65% at the highest dose tested (10 μM). Control experiments demonstrated that **11b**, **16**, and **17** did not quench the fluorescent by-product formed by enzymatic cleavage of z-DEVD-R110. In addition, these three compounds failed to inhibit in vitro caspase-3/7 protease activity indicating their mechanism of action was on impeding enzyme induction.

These results highlight several interesting points. First, a



Scheme 4. (i) H_2 (1 atm), 5% Pd/C, MeOH; (ii) **10**, HBTU, *i*-Pr$_2$EtN, DCM, 85% for **16** over two steps, 81% for **17**.



Graph 1. Inhibition of etoposide-induced caspase-3/7 induction in U937 cells by PDTC (▼), **11b** (▲), **16** (■) and **17** (●). Cells (4×10^4 cells/well) were incubated in the presence of etoposide (40 μM) and in the presence or absence of various concentrations of test compounds for 4.5 h. Caspase-3/7 protease activity was determined using an Apo-ONE Homogeneous Caspase-3/7 Assay kit that employs z-DEVD-R110 as the substrate. Concentration-percent inhibition curves for each test compound were generated using a sigmoidal dose-response with variable slope nonlinear regression analysis ($R^2>0.93$). From these curves, EC_{50} values were determined using GraphPad Prism®.

hydroxy group on the phenethyl portion of the molecules was detrimental to activity. This SAR diverges from that reported for **1**, where derivatives with hydroxy groups on the cinnamate portion of the molecules were most protective.[10b] However, conversion of the hydroxy group on the phenethyl portion of **2a** to an ether (e.g. methyl or *tert*-butyl) resulted in active compounds. Furthermore, the aliphatic methoxy group is not necessary for caspase induction inhibitory activity. The limited maximum inhibition (at 10 μM) observed for **11b** (50%), **16** (58%) and **17** (65%), as compared with PDTC (>95%), might be the result of cellular toxicity caused by the test compounds via non-caspase-3/7 dependent mechanisms resulting in fewer cells undergoing etoposide-induced apoptosis via caspase-3/7 induction.[27] Additional studies will be necessary to determine the precise reasons for these observed effects. Likewise, additional SAR may reveal compounds that are able to more effectively inhibit caspase induction than those reported herein.

3. Conclusions

The methodology described in this communication provided a convenient means for assembling the natural product **2a**, its enantiomer **2b** and several derivatives. It also provides confirmation of the stereochemistry originally assigned to the natural product. The synthesis featured the utilization of an enantioselective catalytic boron-mediated reduction of an α-bromoketone in the key transformation. In addition, **2a** was inactive at inhibiting etoposide-induced caspase induction in U937 cells contrary to a previous report. But, several derivatives (**11b**, **16** and **17**) were found to have activity. The preliminary SAR revealed that the hydroxy group on the phenethyl portion of the molecules is detrimental to activity, replacement of this substituent with an ether resulted in activity and the aliphatic methoxy group is not necessary for caspase induction inhibitory activity.

4. Experimental

4.1. General experimental procedures

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used without further purification. The NMR spectra were obtained using a Bruker 400 MHz, Varian 400 MHz or Varian 500 MHz spectrometer. All ^{1}H NMR spectra are reported in δ units ppm and are reference to tetramethylsilane (TMS) if conducted in $CDCl_3$ or to the central line of the quintet at 3.30 ppm for samples in CD_3OD. All ^{13}C NMR spectra are reported in δ units ppm and are reference to the central line of the triplet at 77.23 ppm if conducted in $CDCl_3$ or to the

central line of the septet at 49.0 ppm for samples in CD_3OD. Column chromatography was performed on silica gel (Merck, grade 60, 230–400 mesh) or utilizing a Combi-Flash Sg 100c separation system (ISCO) with RediSep disposable silica gel columns (ISCO). Optical rotations were measured using an AUTOPOL IV digital polarimeter (Rudolph Research Analytical, NJ). High-resolution mass spectra were obtained by using a SX-102A mass spectrometer (JEOL USA, Inc., Peabody, MA) or a LCT mass spectrometer (Micromass Inc., Beverly, MA). All compounds, unless otherwise noted, are ≥95% pure as determined by ^{1}H NMR.

4.1.1. 4-*tert*-Butyldimethylsiloxyacetophenone (6a). A round-bottom flask was charged with 4-hydroxyacetophenone (**5**, 2.5 g, 18.36 mmol), imidazole (3.09 g, 45.35 mmol), and DMF (20 mL). After cooling the solution to 0°C, *tert*-butyldimethylsilyl chloride (3.32 g, 22.03 mmol) was added in one portion. After stirring at 0°C for 15 min, the solution was maintained at room temperature for 1 h. The solution was diluted with ethyl acetate (100 mL) and then washed sequentially with 1N sodium hydroxide (50 mL), water (3×50 mL) and brine (40 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated to give a colorless oil. The oil was purified by column chromatography on silica gel using hexane/ethyl acetate (90:10) as eluant to give **6a** (4.51 g, 98% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.24 (s, 6H), 0.99 (s, 9H), 2.55 (s, 3H), 6.87 (d, 2H, J=8.8 Hz), 7.88 (d, 2H, J=8.8 Hz).

4.1.2. 4-*tert*-Butoxyacetophenone (6b). The isobutylene gas (ca. 14 g, 250 mmol) was condensed and added dropwise to a mixture of 4-hydroxyacetophenone (6.8 g, 50 mmol) in dichloromethane (100 mL) containing a few drops of concentrated sulfuric acid. The reaction mixture was stirred overnight at room temperature in a two-necked flask with a condenser filled with dry ice. After dilution with 50 mL of dichloromethane, the mixture was washed with water (2×60 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product was purified by chromatography on silica gel using hexane/ethyl acetate (80:20) as eluant to give **6b** (6.1 g, 63% yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42 (s, 9H), 2.57 (s, 3H), 7.03 (d, 2H, J=9.0 Hz), 7.90 (d, 2H, J=9.0 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 26.61 (CH_3), 29.13 (3×CH_3), 79.84 (C), 122.54 (2×CH), 130.02 (2×CH), 132.02 (C), 160.62 (C), 197.35 (C); FT-IR (film, ν_{max}, cm^{-1}): 2979s, 2935w, 1679s, 1598s, 1504m, 1365s, 1255s, 1162s, 1051s, 957w, 897m, 840w; HREIMS [M]$^+$: 193.1233 (calcd for $[C_{12}H_{16}O_2]^+$, 193.1228).

4.1.3. 2′-Bromo-4-*tert*-butyldimethylsiloxyacetophenone (4a). A Schlenk flask, under argon, was charged with **6a** (1.0 g, 4 mmol) and dichloromethane (8 mL). The solution was cooled to 0°C and then diisopropylethylamine (0.88 mL, 5.06 mmol) was added followed by trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.88 mL, 4.86 mmol). The resulting solution was maintained at 0°C for 30 min. Next, *N*-bromosuccinimide (0.854 g, 4.8 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated to give a pale yellow oily solid. This material was purified by column chromatography on silica gel using hexane/ethyl acetate (95:5) as eluant to give **4a** (1.28 g, 97% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.27 (s, 6H), 1.00 (s, 9H), 4.42 (s, 2H), 6.91 (d, 2H, J=8.8 Hz), 7.93 (d, 2H, J=8.8 Hz).

4.1.4. 2′-Bromo-4-*tert*-butoxyacetophenone (4b). Prepared using the procedure described in Section 4.1.3. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (s, 9H), 4.42 (s, 2H), 7.05 (d, 2H, J=9.0 Hz), 7.93 (d, 2H, J=9.0 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 29.13 (3×CH_3), 30.96 (CH_2), 80.18 (C),122.24 (2×CH), 128.35 (C), 130.82 (2×CH), 161.53 (C), 190.40 (C); FT-IR (film, ν_{max}, cm^{-1}): 2978s, 2939w, 1676s, 1597s, 1504m, 1283s, 1257s, 1160s, 1051s, 900m, 848m; HREIMS [M]$^+$: 270.0251 (calcd for $[C_{12}H_{15}BrO_2]^+$, 270.0255).

4.1.5. (*S*)-2-Bromo-1-(4-*tert*-butyldimethylsiloxy)phenylethanol (7a). To a stirred solution of (*S*)-2 methyl-CBS-oxazaborolidine (1.0 M in toluene, 0.1 mL, 0.1 mmol) and borane–methyl sulfide (2.0 M in THF, 0.05 mL, 0.1 mmol) in anhydrous THF (2 mL) were added simultaneously a solution of the α-bromoketone **4a** in THF (1 mL) and borane–methyl sulfide (2.0 M in THF, 0.33 mL, 0.66 mmol) at 0°C under argon over a period of 20 min. After addition, the reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction solution was cooled to 0°C before 1 mL of methanol was added carefully (CAUTION: gas evolution!!). The reaction mixture was then concentrated in vacuo (Me_2S was trapped and oxidized with household bleach) and the residue dissolved in 20 mL of ethyl acetate. The solution was washed with 1N HCl (3×5 mL) and water (2×5 mL), dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (90:10) as eluant to give 293 mg of **7a** as a colorless oil (89%). $[\alpha]_D^{24.4}$=+26.4° (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.20 (s, 6H), 0.99 (s, 9H), 2.59 (s, 1H), 3.54 (q, 1H, J=10.0 Hz), 3.61 (dd, 1H, J=3.2, 10.0 Hz), 4.88 (dd, 1H, J=3.2, 10.0 Hz), 6.84 (d, 2H, J=8.4 Hz), 7.24 (d, 2H, J=8.4 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ −4.21 (2×CH_3–Si), 22.20 (C), 29.66 (3×CH_3), 44.36 (CH_2), 77.61 (CH), 124.28 (2×CH), 131.17 (2×CH), 136.96 (C), 159.89 (C); FT-IR (film, ν_{max}, cm^{-1}): 3391m (br), 2957s, 2930s, 2858s, 1608s, 1510s, 1264s, 916s, 841s, 781s; HREIMS [M]$^+$: 330.0654 (calcd for $[C_{14}H_{23}BrO_2Si]^+$, 330.0651).

4.1.6. (*S*)-2-Bromo-1-(4-*tert*-butoxy)phenylethanol (7b). Prepared using the procedure described in Section 4.1.5. $[\alpha]_D^{23.9}$=+24.0° (*c* 0.5, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34 (s, 9H), 2.62 (d, 1H, J=3.0 Hz), 3.54 (dd, 1H, J=9.0, 10.5 Hz), 3.62 (dd, 1H, J=3.0, 10.0 Hz), 4.89 (dt, 1H, J=3.0, 9.0 Hz), 6.99 (d, 2H, J=8.5 Hz), 7.28 (d, 2H, J=8.5 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 29.04 (3×CH_3), 40.50 (CH_2), 73.80 (CH), 78.94 (C), 124.40 (2×CH), 126.79 (2×CH), 135.17 (C), 155.82 (C); FT-IR (film, ν_{max}, cm^{-1}): 3421s (br), 2977s, 2933w, 1606m, 1506s, 1367m,1238s, 1160s, 1070m, 895s, 854m; HREIMS [M]$^+$: 272.0413 (calcd for $[C_{12}H_{17}BrO_2]^+$, 272.0412).

4.1.7. (*S*)-2-Azido-1-(4-*tert*-butyldimethylsiloxy)phenylethanol (8a). A mixture of the bromohydrin **7a** (132 mg,

0.4 mmol), sodium azide (52 mg, 0.8 mmol), tetrabutylammonium bromide (80 mg, 0.24 mmol) and water (1.5 mL) was stirred at 40°C for 2 days and then extracted with ethyl acetate (30 mL). The organic layer was washed with water (2×8 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate (90:10) as eluant to afford 70 mg of **8a** as a colorless oil (60%). $[\alpha]_D^{25.9}$=+46.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.20 (s, 6H), 0.99 (s, 9H), 2.26 (s, 1H), 3.45 (m, 2H), 4.83 (m, 1H), 6.64 (d, 2H, *J*=8.4 Hz), 7.23 (d, 2H, *J*=8.4 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ −4.22 (2×CH_3–Si), 18.41 (C), 25.86 (3×CH_3), 58.30 (CH_2), 73.36 (CH), 120.50 (2×CH), 127.34 (2×CH), 133.44 (C), 156.01 (C); FT-IR (film, ν_{max}, cm^{-1}): 3404m (br), 2957m, 2930m, 2859m, 2105s, 1608m, 1511s, 1263s, 915s, 840s, 781s; HREIMS $[M]^+$: 293.1564 (calcd for $[C_{14}H_{23}N_3O_2Si]^+$, 293.1560).

4.1.8. (*S*)-2-Azido-1-(4-*tert*-butoxy)phenylethanol (8b). Prepared using the procedure described in Section 4.1.7. $[\alpha]_D^{24.0}$=+71.6° (*c* 0.5, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34 (s, 9H), 2.34 (s, 1H), 3.42 (dd, 1H, *J*=3.5, 12.5 Hz), 3.48 (dd, 1H, *J*=8.0, 12.5 Hz), 4.89 (dt, 1H, *J*=3.5, 8.0 Hz), 6.99 (d, 2H, *J*=8.5 Hz), 7.26 (d, 2H, *J*=8.5 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 29.03 (3×CH_3), 58.29 (CH_2), 73.34 (CH), 78.94 (C), 124.46 (2×CH), 126.75 (2×CH), 135.51 (C), 155.71 (C); FT-IR (film, ν_{max}, cm^{-1}): 3427s (br), 2978s, 2933w, 2104s, 1608m, 1507s, 1367m, 1238s, 1161s, 1070m, 894s, 856m; HREIMS $[M]^+$: 235.1313 (calcd for $[C_{12}H_{17}N_3O_2]^+$, 235.1321).

4.1.9. (*S*)-1-(2-Azido-1-methoxy)ethyl-4-*tert*-butyldimethylsiloxybenzene (9a). *Method A*. To a stirred solution of the azido alcohol **8a** (30 mg, 0.1 mmol) in THF (1.5 mL) was added sodium hydride (95%, 5 mg, 0.2 mmol) at room temperature under argon. The mixture was stirred for 15 min and then iodomethane (0.02 mL, 0.3 mmol) was added. Stirring was continued for another 1 h before the reaction mixture was diluted with 20 mL of ethyl acetate. The mixture was washed with 1N HCl (5 mL), saturated $NaHCO_3$ (5 mL), and brine (5 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate (95:5) as eluant to give 15–27 mg of **9a** as a colorless oil (50–90%).

Method B. A mixture of the azido alcohol **8a** (29 mg, 0.1 mmol), silver (I) oxide (24 mg, 0.11 mmol), iodomethane (1 mL) and acetonitrile (2 mL) was stirred at 68°C for 2 days. The solid was then filtered and washed with ethyl acetate (2×3 mL). After removal of the solvents, the residue was purified by column chromatography on silica gel using hexane/ethyl acetate (90:10) as eluant to give 22 mg of **9a** as a colorless oil (72%). $[\alpha]_D^{24.6}$=+95.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.21 (s, 6H), 0.99 (s, 9H), 3.19 (dd, 1H, *J*=3.2, 12.4 Hz), 3.29 (s, 3H), 3.47 (dd, 1H, *J*=8.4, 12.4 Hz), 4.30 (dd, 1H, *J*=3.2, 8.4 Hz), 6.84 (d, 2H, *J*=8.4 Hz), 7.18 (d, 2H, *J*=8.4 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ −4.22 (2×CH_3–Si), 18.38 (C), 25.85 (3×CH_3), 56.80 (CH_2), 51.90 (CH_3), 82.92 (CH), 120.43 (2×CH), 128.07 (2×CH), 131.34 (C), 156.03 (C); FT-IR (film, ν_{max}, cm^{-1}): 2956s, 2930s, 2859s, 2102s, 1608m, 1510s, 1256s, 1110s, 915s, 840s, 782m; HREIMS $[M]^+$: 307.1719 (calcd for $[C_{15}H_{25}N_3O_2Si]^+$, 307.1716).

4.1.10. (*S*)-1-(2-Azido-1-methoxy)ethyl-4-*tert*-butoxybenzene (9b).[28] Prepared using the procedure described in Section 4.1.9, Method B. $[\alpha]_D^{24.0}$=+120.0° (*c* 0.5, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35 (s, 9H), 3.20 (dd, 1H, *J*=4.0, 13.0 Hz), 2.30 (s, 3H), 3.48 (dd, 1H, *J*=8.5, 13.0 Hz), 4.32 (dd, 1H, *J*=4.0, 8.5 Hz), 6.99 (d, 2H, *J*=9.0 Hz), 7.20 (d, 2H, *J*=9.0 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 29.06 (3×CH_3), 56.79 (CH_3), 57.02 (CH_2), 78.83 (C), 82.95 (CH), 124.37 (2×CH), 127.45 (2×CH), 133.37 (C), 155.83 (C); FT-IR (film, ν_{max}, cm^{-1}): 2979s, 2933w, 2102s, 1608m, 1506s, 1366m,1238s, 1162s, 1110s, 896s, 859m.

4.1.11. (*S*)-1-(2-Amino-1-methoxy)ethyl-4-*tert*-butyldimethylsiloxybenzene (3a). A mixture of azido methyl ether **9a** (31 mg, 0.1 mmol), 5% Pd/C (30 mg) and methanol (1.5 mL) was stirred for 1.5 h at room temperature under hydrogen (1 atm), then diluted with methanol (20 mL) and 1 mL of 2.0 M ammonia in ethanol. The catalyst was removed by filtration and the filtrate concentrated. The residue was dissolved in ethyl acetate (20 mL) and dried over anhydrous Na_2SO_4, filtered and evaporated to afford 30 mg of crude product **3a**, which was used without further purification.

4.1.12. (*S*)-1-(2-Amino-1-methoxy)ethyl-4-*tert*-butoxybenzene (3b). A mixture of azido methyl ether **9b** (125 mg, 0.5 mmol), 5% Pd/C (50 mg) and methanol (6 mL) was stirred for 2 h at room temperature under hydrogen (1 atm), then diluted with methanol (20 mL) and 1 mL of 2.0 M ammonia in ethanol. The catalyst was removed by filtration and the filtrate concentrated. The residue was dissolved in ethyl acetate (20 mL) and dried over anhydrous Na_2SO_4, filtered and evaporated to afford 111 mg of crude product **3b**, which was used without further purification.

4.1.13. 4-Hydroxy-3-methoxycinnamic acid (10). A round-bottom flask was charged with ethyl 4-hydroxy-3-methoxycinnamate (50 mg, 0.225 mmol), THF (2 mL), and 2N sodium hydroxide (5 mL). The reaction mixture was stirred at room temperature for 8 h and then made acidic with concentrated hydrochloric acid. The mixture was extracted with ethyl acetate (3×10 mL). The extracts were combined, washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to give a pale yellow solid. The solid was purified by column chromatography on silica gel using chloroform/ethyl acetate/acetic acid (10:1:0.1) as eluant to give **10** (43 mg, 98% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 3.90 (s, 3H), 4.99 (bs, 2H), 6.32 (d, 1H, *J*=16 Hz), 6.82 (d, 1H, *J*=8.2 Hz), 7.07 (dd, 1H, *J*=1.6, 8.2 Hz), 7.18 (d, 1H, *J*=1.6 Hz), 7.61 (d, 1H, *J*=16 Hz).

4.1.14. (*S*)-3-(4-Hydroxy-3-methoxyphenyl)-*N*-[2-(4-*tert*-butyldimethylsiloxyphenyl)-2-methoxyethyl]acrylamide (11a). A mixture of the cinnamic acid **10** (20 mg, 0.1 mmol), HBTU (34 mg, 0.09 mmol), *i*-Pr_2NEt (0.04 mL, 0.2 mmol) and the amino methyl ether **3a**

(10 mg, 0.03 mmol) in 1 mL of CH_2Cl_2 was stirred at room temperature for 1 h. The reaction mixture was diluted with 20 mL of ethyl acetate before being washed sequentially with 1N HCl (2×5 mL), saturated $NaHCO_3$ (2×5 mL) and brine (8 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (60:40) as eluant to give 14 mg of **11a** as a pale yellow oil (86% for two steps). $[\alpha]_D^{24.5}=-1.6°$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.21 (s, 6H), 0.99 (s, 9H), 3.24 (s, 3H), 3.30 (m, 1H), 3.84 (m, 1H), 3.92 (s, 3H), 4.26 (dd, 1H, *J*=4.0, 8.8 Hz), 5.93 (s, 1H), 6.03 (m, 1H), 6.26 (d, 1H, *J*=15.6 Hz), 6.84 (d, 2H, *J*=8.8 Hz), 6.91 (d, 1H, *J*=7.6 Hz), 7.00 (s, 1H), 7.07 (d, 1H, *J*=7.6 Hz), 7.19 (d, 2H, *J*=8.8 Hz), 7.56 (d, 1H, *J*=15.6 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ −4.19 (2×CH_3−Si), 18.40 (C), 25.87 (3×CH_3), 45.96 (CH_2), 56.15 (CH_3), 56.80 (CH_3), 82.19 (CH), 109.84 (CH), 114.91 (CH), 118.45 (CH), 120.47 (2×CH), 122.46 (CH), 127.62 (C), 128.12 (2×CH), 131.81 (C), 141.35 (CH), 146.90 (C), 147.57 (C), 155.85 (C), 166.23 (C); FT-IR (KBr, ν_{max}, cm^{-1}): 3412m (br), 2955m, 2933m, 2857m, 1729m, 1659m, 1606m, 1510s, 1261s, 1121s, 915m, 841m, 782m; HRESMS $[M+H]^+$: 458.2369 (calcd for $[C_{25}H_{35}NO_5Si+H]^+$, 458.2363).

4.1.15. (*S*)-3-(4-Hydroxy-3-methoxyphenyl)-*N*-[2-(4-*tert*-butyoxyphenyl)-2-methoxyethyl]acrylamide (11b). Prepared using the procedure described in Section 4.1.14. $[\alpha]_D^{23.9}=-27.2°$ (*c* 0.5, MeOH); ^1H NMR (500 MHz, $CDCl_3$): δ 1.35 (s, 9H), 3.26 (s, 3H), 3.30 (m, 1H), 3.84 (m, 1H), 3.93 (s, 3H), 4.28 (dd, 1H, *J*=4.0, 9.0 Hz), 5.83 (s, 1H), 6.00 (m, 1H), 6.26 (d, 1H, *J*=16.0 Hz), 6.91 (d, 1H, *J*=8.5 Hz), 6.98 (d, 2H, *J*=9.0 Hz), 7.00 (d, 1H, *J*=2.0 Hz), 7.07 (dd, 1H, *J*=2.0, 8.5 Hz), 7.22 (d, 2H, *J*=9.0 Hz), 7.55 (d, 1H, *J*=16.0 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 29.09 (3×CH_3), 45.97 (CH_2), 56.16 (CH_3), 56.93 (CH_3), 78.80 (C), 82.24 (CH), 109.76 (CH), 114.93 (CH), 118.43 (CH), 122.46 (CH), 124.35 (2×CH), 127.49 (2×CH), 127.62 (C), 133.87 (C), 141.38 (CH), 146.93 (C), 147.61 (C), 155.63 (C), 166.26 (C); FT-IR (film, ν_{max}, cm^{-1}): 3512–3057s (br), 2979m, 2935m, 2827w, 1658s, 1605s, 1514s, 1268m,1205s, 1162s, 1110s, 895w, 847m, 737s; HRESMS $[M+H]^+$: 400.2113 (calcd for $[C_{23}H_{29}NO_5+H]^+$, 400.2124).

4.1.16. (*S*)-3-(4-Hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide (2a). A solution of 1.0 M tetrabutylammonium fluoride (1 mL) in THF was added to **11a** (14 mg, 0.03 mmol) at room temperature. The mixture was stirred for 5 min before removal of the solvent. The residue was dissolved in 20 mL of ethyl acetate, washed with 1N HCl (2×5 mL) and brine (10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (40:60) as eluant to give 10 mg of **2a** as a pale yellow oil (97%). $[\alpha]_D^{26.1}=-5.6°$ (*c* 1.0, CH_3OH), literature reference $[\alpha]_D^{25}=-2°$ (*c* 1.0, CH_3OH);[11] ^1H NMR (400 MHz, CD_3OD): δ 3.21 (s, 3H), 3.41 (dd, 1H, *J*=8.4, 14.0 Hz), 3.50 (dd, 1H, *J*=4.8, 14.0 Hz), 3.88 (s, 3H), 4.25 (dd, 1H, *J*=4.4, 8.0 Hz), 6.47 (d, 1H, *J*=15.6 Hz), 6.79 (d, 1H, *J*=8.8 Hz), 6.80 (d, 1H, *J*=8.8 Hz), 7.03 (dd, 1H, *J*=2.4, 8.4 Hz), 7.13 (d, 1H, *J*=2.4 Hz), 7.17 (d, 2H, *J*=8.8 Hz), 7.44 (d, 1H, *J*=15.6 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 46.09 (CH_2), 56.15 (CH_3), 56.80 (CH_3), 82.16 (CH), 109.74 (CH), 114.90 (CH), 115.74 (2×CH), 118.27 (CH), 122.49 (CH), 127.99 (C), 128.35 (2×CH), 131.13 (C), 141.60 (CH), 146.88 (C), 147.61 (C), 155.96 (C), 166.37 (C); HRESMS $[M+H]^+$: 344.1491 (calcd for $[C_{19}H_{21}NO_5+H]^+$, 344.1498).

4.1.17. (*R*)-3-(4-Hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide (2b). Prepared using the same procedure as described in Section 4.1.16. $[\alpha]_D^{26.0}=+6.1°$ (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, CD_3OD): δ 3.21 (s, 3H), 3.41 (dd, 1H, *J*=8.4, 14.0 Hz), 3.50 (dd, 1H, *J*=4.8, 14.0 Hz), 3.88 (s, 3H), 4.25 (dd, 1H, *J*=4.4, 8.0 Hz), 6.47 (d, 1H, *J*=15.6 Hz), 6.79 (d, 1H, *J*=8.8 Hz), 6.80 (d, 1H, *J*=8.8 Hz), 7.03 (dd, 1H, *J*=2.4, 8.4 Hz), 7.13 (d, 1H, *J*=2.4 Hz), 7.17 (d, 2H, *J*=8.8 Hz), 7.44 (d, 1H, *J*=15.6 Hz); HRESMS $[M+H]^+$: 344.1494 (calcd for $[C_{19}H_{21}NO_5+H]^+$, 344.1498).

4.1.18. *N*-[2-(4-*tert*-Butyldimethylsiloxyphenyl)-2(*S*)-methoxyethyl]-*N′*-BOC-L-alaninamide (13a). A solution of *N*-Boc-L-alanine (**12**, 19 mg, 0.1 mmol), HBTU (34 mg, 0.09 mmol), *i*-Pr_2NEt (0.06 mL, 0.3 mmol) in 1.1 mL of CH_2Cl_2/DMF (10:1) was added to the amino methyl ether **3a** (10 mg, 0.03 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with 20 mL of ethyl acetate before being washed sequentially with 1N HCl (2×5 mL), saturated $NaHCO_3$ (2×5 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (50:50) as eluant to give 12 mg of **13a** as a colorless oil (88%). ^1H NMR (500 MHz, $CDCl_3$): δ 0.20 (s, 6H), 0.98 (s, 9H), 1.34 (d, 3H, *J*=7.0 Hz), 1.46 (s, 9H), 3.22 (s, 3H), 3.22 (m, 1H), 3.64 (m, 1H), 4.17 (m, 2H), 5.06 (bs, 1H), 6.40 (m, 1H), 6.82 (d, 2H, *J*=8.5 Hz), 7.15 (d, 2H, *J*=8.5 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ −4.21 (2×CH_3−Si), 18.39 (C), 18.96 (CH_3), 25.85 (3×CH_3), 28.55 (3×CH_3), 45.75 (CH_2), 50.29 (CH), 56.82 (CH_3), 80.17 (C), 81.94 (CH), 120.34 (2×CH), 128.04 (2×CH), 131.66 (C), 155.59 (C), 155.83 (C), 172.66 (C); FT-IR (film, ν_{max}, cm^{-1}): 3319s (br), 2957s, 2930s, 2858s, 1716s, 1660s, 1608m, 1510s, 1366s, 1253s, 1168s, 916s, 841s, 781m; HRESMS $[M+Na]^+$: 475.2601 (calcd for $[C_{23}H_{40}N_2O_5Si+Na]^+$, 475.2604).

4.1.19. 2-(4-*tert*-Butyldimethylsiloxyphenyl)-2(*R*)-methoxyethyl]-*N′*-BOC-L-alaninamide (13b). A solution of *N*-Boc-L-alanine (**12**, 23 mg, 0.12 mmol), HBTU (38 mg, 0.10 mmol), *i*-Pr_2NEt (0.08 mL, 0.3 mmol) in 1.1 mL of CH_2Cl_2/DMF (10:1) was added to the amino methyl ether **3b** (12 mg, 0.04 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with 20 mL of ethyl acetate before being washed sequentially with 1N HCl (2×5 mL), saturated $NaHCO_3$ (2×5 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate (50:50) as eluant to give 16 mg of **13b** as a colorless oil (84%). ^1H NMR (500 MHz, $CDCl_3$): δ 0.20 (s, 6H), 0.98 (s, 9H), 1.36 (d, 3H, *J*=7.0 Hz), 1.46 (s, 9H), 3.18 (m, 1H), 3.22 (s, 3H), 3.68 (m, 1H), 4.17 (m, 2H), 4.96 (bs, 1H), 6.46

(m, 1H), 6.82 (d, 2H, J=8.5 Hz), 7.15 (d, 2H, J=8.5 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ −4.21 (2×CH_3–Si), 18.38 (C), 18.86 (CH_3), 25.86 (3×CH_3), 28.52 (3×CH_3), 45.73 (CH_2), 50.50 (CH), 56.83 (CH_3), 80.24 (C), 82.03 (CH), 120.32 (2×CH), 128.04 (2×CH), 131.65 (C), 155.54 (C), 155.81 (C), 172.64 (C); FT-IR (film, ν_{max}, cm^{-1}): 3320s (br), 2957s, 2930s, 2858s, 1716s, 1660s, 1608m, 1509s, 1366s, 1253s, 1168s, 916s, 841s, 781m; HRESMS $[M+Na]^+$: 475.2581 (calcd for $[C_{23}H_{40}N_2O_5Si+Na]^+$, 475.2604).

4.1.20. (*S*)-1-(2-Azido-1-methoxy)ethyl(4-methoxy)benzene (14a). To a stirred solution of the azido alcohol **8a** (110 mg, 0.37 mmol) in THF (5 mL) was added sodium hydride (95%, 40 mg, 1.6 mmol) at room temperature under argon. The mixture was stirred for 15 min and then iodomethane (0.08 mL, 1.2 mmol) was added. Stirring was continued for an additional 2 h and then the reaction mixture was diluted with 40 mL of ethyl acetate. The mixture was washed with 1N HCl (10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate (90:10) as eluant to give **14a** (50 mg, 43% yield) as a colorless oil. $[\alpha]_D^{22.9}$=+86.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.18 (dd, 1H, J=4.0, 13.0 Hz), 3.28 (s, 3H), 3.48 (dd, 1H, J=8.5, 13.0 Hz), 3.81 (s, CH_3), 4.31 (dd, 1H, J=4.0, 8.5 Hz), 6.90 (d, 2H, J=9.0 Hz), 7.24 (d, 2H, J=9.0 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 55.51 (CH_3), 56.83 (CH_3), 56.90 (CH_2), 82.86 (CH), 114.32 (2×CH), 128.16 (2×CH), 130.77 (C), 159.94 (C); FT-IR (film, ν_{max}, cm^{-1}): 2991w, 2934m, 2835w, 2102s, 1612m, 1513s, 1249s, 1107s, 1033s, 832m; HRCIMS $[M+NH_4]^+$: 225.1360 (calcd for $[C_{10}H_{13}N_3O+NH_4]^+$, 225.1351).

4.1.21. (*S*)-1-(2-Amino-1-methoxy)ethyl(4-methoxy)benzene (14b). A mixture of azido methyl ether **14a** (30 mg, 0.15 mmol), 5% Pd/C (30 mg) and methanol (2 mL) was stirred for 3 h at room temperature under hydrogen (1 atm), then diluted with methanol (20 mL) and 1 mL of 2.0 M ammonia in ethanol. The catalyst was removed by filtration and the filtrate concentrated. The residue was dissolved in ethyl acetate (20 mL) and dried over anhydrous Na_2SO_4, filtered and evaporated to afford 24 mg of crude product **14b**, which was used without further purification.

4.1.22. (*S*)-*N*-[2-(4-*tert*-Butoxyphenyl)-2-methoxyethyl]-3-(4-hydroxy-3-methoxyphenyl)acrylamide (16). Prepared using the procedure described in Section 4.1.14. $[\alpha]_D^{22.2}$=−24.0° (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.26 (s, 3H), 3.30 (m, 1H), 3.82 (s, 3H), 3.83 (m, 1H), 3.93 (s, 3H), 4.27 (dd, 1H, J=4.0, 9.0 Hz), 5.81 (s, 1H), 5.99 (m, 1H), 6.26 (d, 1H, J=15.0 Hz), 6.91 (d, 1H, J=8.0 Hz), 6.92 (d, 2H, J=8.5 Hz), 7.01 (d, 1H, J=2.0 Hz), 7.07 (dd, 1H, J=2.0, 8.0 Hz), 7.26 (d, 2H, J=8.5 Hz), 7.56 (d, 1H, J=15.0 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 45.97 (CH_2), 55.49 (CH_3), 56.15 (CH_3), 56.79 (CH_3), 82.13 (CH), 109.79 (CH), 114.24 (2×CH), 114.93 (CH), 118.41 (CH), 122.44 (CH), 127.59 (C), 128.18 (2×CH), 131.23 (C), 141.38 (CH), 146.93 (C), 147.61 (C), 159.76 (C), 166.26 (C); FT-IR (film, ν_{max}, cm^{-1}): 3600–3100s (br), 2979m, 2932m, 2858m, 1647s, 1516s, 1272s,1205s, 1162m, 1115s, 895w, 844m; HRESMS $[M+H]^+$: 358.1649 (calcd for $[C_{20}H_{23}NO_5+H]^+$, 358.1654).

4.1.23. 3-(4-Hydroxy-3-methoxyphenyl)-*N*-[2-(4-methoxyphenyl)ethyl]acrylamide (17). Prepared using the procedure described in Section 4.1.14. ^{1}H NMR [500 MHz, $CDCl_3$(CD_3OD)]: δ 2.82 (t, 2H, J=7.0 Hz), 3.57 (t, 2H, J=7.0 Hz), 3.81 (s, 3H), 3.91 (s, 3H), 6.23 (d, 1H, J=15.5 Hz), 6.85 (d, 1H, J=8.0 Hz), 6.87 (d, 2H, J=9.0 Hz), 7.01 (d, 1H, J=2.0 Hz), 7.03 (dd, 1H, J=2.0, 8.0 Hz), 7.26 (d, 2H, J=9.0 Hz), 7.49 (d, 1H, J=15.5 Hz); ^{13}C NMR [100.5 MHz, $CDCl_3$(acetone-d_6)]: δ 34.77 (CH_2), 41.02 (CH_2), 55.11 (CH_3), 55.81 (CH_3), 109.88 (CH), 113.91 (2×CH), 114.92 (CH), 118.40 (CH), 121.98 (CH), 127.34 (C), 129.68 (2×CH), 131.09 (C), 140.54 (CH), 147.12 (C), 147.66 (C), 158.20 (C), 166.38 (C); FT-IR (film, ν_{max}, cm^{-1}): 3736–3100s (br), 2930m, 2850m, 1659s, 1615s, 1514s, 1460m, 1275s,1209s, 1123m, 1030s, 846s; HRESMS $[M+H]^+$: 328.1545 (calcd for $[C_{19}H_{21}NO_4+H]^+$, 328.1549).

4.2. Etoposide-induced Caspase-3/7 induction assay

A 20 μL aliquot of U937 cells (4×10^4 cells/well; obtained from American Tissue Culture Co.) in growth media (DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin–streptomycin, 2 mM glutamine, 1 mM sodium pyruvate, 25 mM HEPES, 1.5 g/L sodium bicarbonate) was added to a 384-well plate. Etoposide (40 μM) was added to the cells in the presence or absence of various concentrations of test compounds (10–0.001 μM) to give a final volume of 50 μL. Stock solutions of compounds were prepared in DMSO at a concentration of 10 mM. The vehicle control contained 0.16% DMSO. Cells were incubated for 4.5 h at 37°C in an incubator containing 5% CO_2–95% air atmosphere. After observing apoptotic cells by microscopy, the caspase-3/7 protease activity was estimated by addition of 50 μL of substrate Z-DEVD-R110 (Rhodamine 110 labeled bis-(*N*-CBZ-L-aspartyl-L-glutamyl-L-valyl-aspartic acid amide; Apo-ONE Homogeneous Caspase-3/7 Assay kit from Promega). Incubation was carried out at room temperature for 1 h and the fluorescent intensity (reported as RFU) was monitored by excitation at 485 nm and emission at 530 nm in a fluorescence plate reader (Molecular Devices). Each sample represents the average of four replicates. Concentration-percent inhibition curves for each test compound were generated using a sigmoidal dose-response with variable slope nonlinear regression analysis (R^2>0.93). From these curves, EC_{50} values were determined using GraphPad Prism®. Ammonium pyrrolidine dithiocarbamate (PDTC; Sigma-Aldrich, St. Louis, MO) was used as a standard caspase induction inhibitor. The activity of PDTC in the assay described herein was lower (EC_{50}=0.51 μM) than reported by Kho et al. (EC_{50}=50 μM).[11]

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