

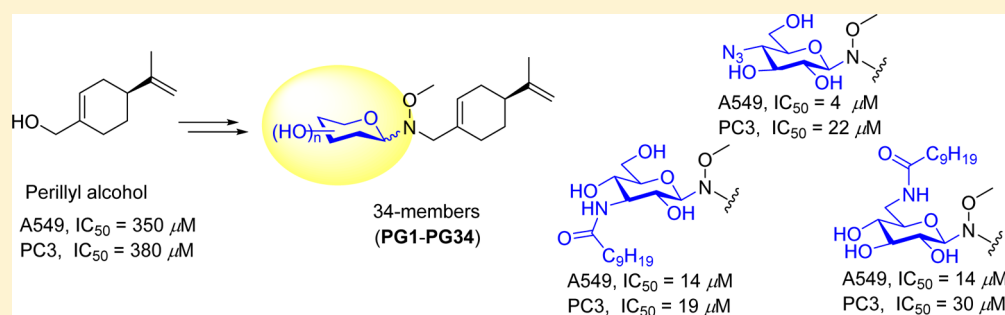
The Identification of Perillyl Alcohol Glycosides with Improved Antiproliferative Activity

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S Supporting Information



ABSTRACT: A facile route to perillyl alcohol (POH) differential glycosylation and the corresponding synthesis of a set of 34 POH glycosides is reported. Subsequent in vitro studies revealed a sugar dependent antiproliferative activity and the inhibition of S6 ribosomal protein phosphorylation as a putative mechanism of representative POH glycosides. The most active glycoside from this cumulative study (4'-azido-D-glucoside, PG9) represents one of the most cytotoxic POH analogues reported to date.

INTRODUCTION

(S)-Perillyl alcohol (POH, also known as *p*-metha-1,8-diene-7-ol or 4-isopropenyl cyclohexene carbinol) is a monoterpene produced by a number of plants, including cherries, lavandin, mints, and celery seeds via oxidative modification of D-limonene.^{1,2} Interest in POH as a potential anticancer agent stems from its ability to cause G1 cancer cell cycle arrest via the putative inhibition of post-translational modification of signal transduction proteins involved in the Ras/MAPK pathway.³ While the fundamental mechanism(s) and/or target(s) of POH remain unclear, POH has been implicated in a range of functions including inhibition of small G protein isoprenylation and induction of proto-oncogenes,⁴ inhibition of Na/K-ATPase,⁵ disruption of hTERT-mTOR-RAPTOR protein complex,⁶ suppression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase synthesis in mammalian cells,^{2,7} as well as inhibition of 4E-BP1(Ser65) phosphorylation and cap-dependent translation.⁸ POH was initially evaluated in phase I and phase II clinical trials for the treatment of a range of cancers (breast, colon, ovarian, and prostate).^{9–16} While these studies revealed POH to be well tolerated at high doses, tumor responses were low. However, more recent clinical studies focused upon intranasal delivery of POH to treat recurrent malignant glioblastoma led to notable tumor regression with high doses of POH.^{17–19} This promising precedent suggests that POH analogues with improved potency and/or drug

properties may serve to reinvigorate the clinical utility of this unique plant metabolite and also potentially offer new probes for further mechanistic interrogation.

The glycosylation of small-molecule-based or natural product-based leads/drugs can often dramatically influence pharmacological properties and ADMET.²⁰ In the context of POH, a small set of naturally occurring POH glycosides (Figure 1; 1a, 2a, 9a, 10a) isolated from *Perilla frutescens* leaves was first reported as aldose reductase inhibitors.^{21–24} A few additional POH glycosides were generated via conventional or enzymatic synthesis,^{21,25–27} (Figure 1; 3a–8a, 11a) and, cumulatively, these studies revealed certain POH glucosides (Figure 1; 1a, 3a) as equipotent to POH against select cancer cell lines in vitro. Yet, studies designed to systematically assess and/or exploit the impact of POH glycosylation are lacking. Neoglycorandomization, a divergent chemoselective glycosylation method, is advantageous in this regard as it offers a rapid strategy for differential glycosylation of a selected target scaffold.²⁸ Herein we report the synthesis and in vitro anticancer activity of a set of 34 distinct POH neoglycosides, several of which displayed improved in vitro anticancer activity over the parent natural product. The best among these (4'-azido-D-glucoside PG9) displayed a striking enhancement in

Received: June 9, 2014

Published: August 14, 2014

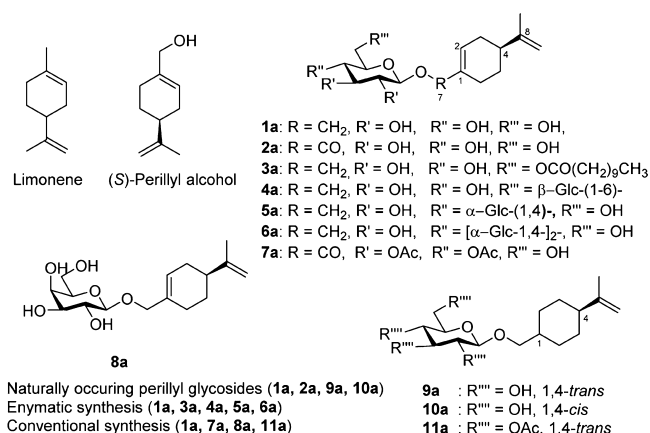


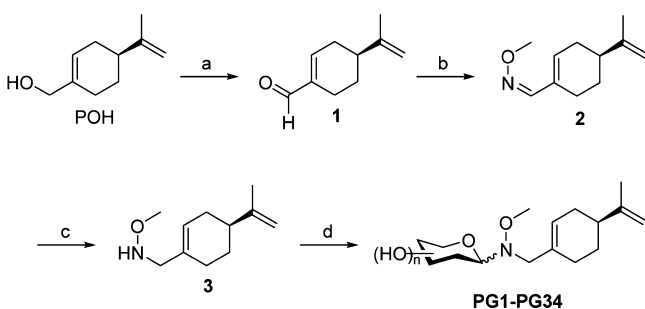
Figure 1. Limonene, perillyl alcohol (POH), and previously reported POH glycosides.

potency (>85-fold against A549 nonsmall cell lung; 15-fold against PC3 prostate) over POH and represents the most active perillyl glycoside reported to date. In contrast to previous reports,⁸ the subsequent study of the influence of POH and representative improved POH glycosides upon 4E-BP1 phosphorylation in A549 cells suggests the antiproliferative effects of the improved POH glycosides may be associated with the inhibition of cap-dependent translation by targeted inhibition of phosphorylation of S6 ribosomal protein rather than 4E-BP1. This study also highlights, for the first time, the compatibility of neoglycosides with Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition.

RESULTS AND DISCUSSION

Perillyl neoglycon **3** was synthesized in three simple steps (Scheme 1). Specifically, POH was oxidized to (*S*)-peril-

Scheme 1. Synthesis of Perillylneoglycon (**3**) and Neoglycosides (PG1–PG34)^a

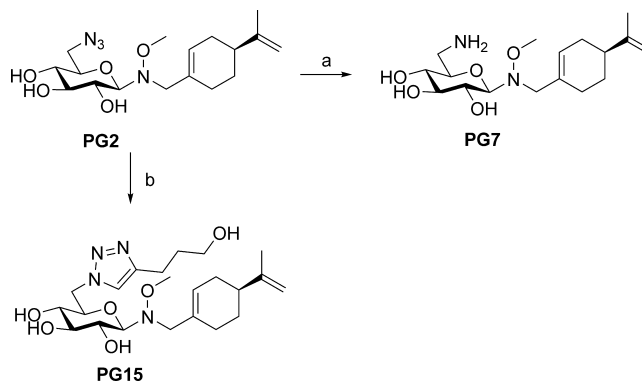


^aReagents and conditions: (a) IBX, HOAc, acetonitrile (88%); (b) MeONH₃Cl, Et₃N, EtOH (84%); (c) NaCNBH₃, HOAc, MeOH (50%); (d) reducing sugar, MeOH:HOAc (5:1), 40 °C (23%–77%).

aldehyde (**1**) using *o*-iodoxybenzoic acid (IBX) and acetic acid.²⁹ Subsequent reductive amination proceeded via formation of oxime **2** with methoxyamine hydrochloride in the presence of triethylamine, followed by reduction with NaCNBH₃ in the presence of acetic acid to provide the requisite neoglycon **3**.³⁰ Optimization of **3** neoglycosylation with D-glucose using a range of general neoglycosylation conditions^{31–33} revealed MeOH:HOAc (5:1) as most effective, providing the corresponding D-glucoside **PG1** in 69% isolated yield and, consistent with prior studies,³⁴ the β-anomer

exclusively. The scope of sugars selected for this study included representative pentoses (D/L-arabinose, **PG3/PG28**; L-ribose, **PG17**; D-xylose, **PG22**), hexoses (D/L-glucose, **PG1/PG6**; 3-O-methyl-D-glucose, **PG4**; L-rhamnose, **PG32**), azidosugars (6-deoxy-6-azido-D-glucose, **PG2**; 3-deoxy-3-azido-D-glucose, **PG8**; 4-deoxy-4-azido-D-glucose, **PG9**; 2-deoxy-2-azido-D-glucose, **PG13**; 4,6-deoxy-4,6-diazido-D-glucose, **PG20**, 4-deoxy-4-azido-L-glucose, **PG24**; 4-deoxy-4-azido-D-xylose, **PG34**), fluorosugars (3-deoxy-3-fluoro-D-glucose, **PG18**; 4-deoxy-4-fluoro-D-glucose, **PG21**; 2-deoxy-2-fluoro-D-glucose, **PG29**; 2-deoxy-2-fluoro-D-mannose, **PG30**) N-acyl sugars (N-acetyl-D-galactosamine, **PG5**; N-acetylmuramic acid, **PG12**; streptozocin, **PG16**; 3-N-decanoyl-D-glucosamine, **PG23**; 6-N-decanoyl-D-glucosamine, **PG26**; 3-N-allyloxycarbonyl-D-glucosamine, **PG27**; 2-N-allyloxycarbonyl-D-glucosamine, **PG31**; 6-N-allyloxycarbonyl-D-glucosamine, **PG33**), an acid-bearing sugar (D-glucuronic acid, **PG25**), and a disaccharide (D-cellobiose, **PG14**) with a bias toward glucosides and acyl glucosides based upon the previously reported active glycosides (**1a** and **3a**). The inclusion of azidosugars served as a starting point for subsequent divergence via chemoselective modification [via Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition]^{35,36} or selective reduction to afford the corresponding aminosugar conjugates (Scheme 2).^{30,32,37–39} A library of 34 distinct

Scheme 2. Divergent Transformations of Azidosugar-Bearing Neoglycoside **PG2**^a



^aReagent and conditions: (a) PMe₃ in THF, overnight, (46%); (b) 4-pentyn-1-ol, CuI, Et₃N, ACN, overnight, (82%).

neoglycosides (**PG1–PG34**) were synthesized (Figure S1 and Table S1 in Supporting Information) in good to excellent yields (23–82%), with the β-anomer as predominate product in most cases, consistent with previous studies.^{40–49} For aminosugar conjugates, azidosugar glycosides (**PG2**, **PG8**, **PG9**, **PG13**) were readily reduced to their corresponding aminosugar counterparts (**PG7**, **PG10**, **PG11**, **PG19**) in the presence of PMe₃ (1.0 M in THF), with yields ranging from 29 to 46%. To assess the compatibility of neoglycosides with Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition and the potential impact of sugar triazole substitution upon POH bioactivity, the 6'-azido perillyl glucoside was also converted to the corresponding 6'-triazole perillyl glucoside **PG15** via Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition in 82% yield (Scheme 2).

The anticancer properties of POH, **1**, **2**, **3**, and **PG1–PG34** against two human cancer cell lines (nonsmall-cell lung A549 and prostate PC3) was first assessed via percent inhibition of cell viability at a single dose of 250 μM (Figure 3). Compounds which displayed >40% inhibition in one or both cell lines

(oxime 2, PG2, PG9, PG13, PG16, PG20, PG22, PG23, PG24, PG26, PG31, PG33, PG34) from the single dose analysis were subsequently subjected to a full dose study for IC_{50} determination along with the parental POH (Figure 2, 4).

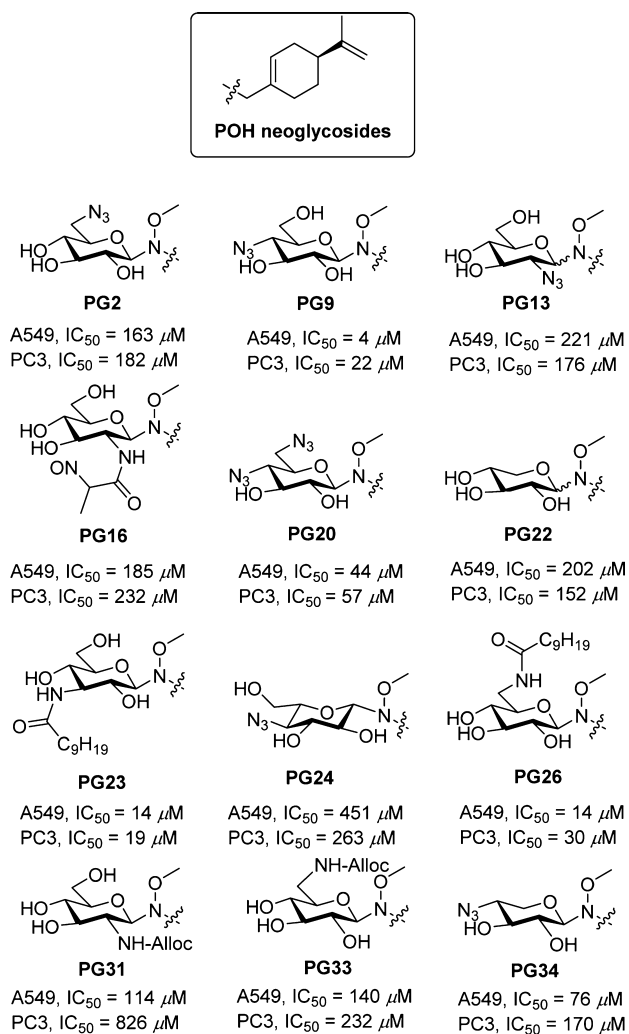


Figure 2. Structures of the most antiproliferative POH neoglycosides against A549 and PC3 cell lines.

This broad analysis revealed the following key observational trends. First, substitutions of key sugar hydroxyls with an azide present the greatest improvements. For example, while D-glucoside PG1 was relatively inactive ($>500 \mu$ M in both cell lines), C6' (PG2: 163 μ M, A549; 182 μ M, PC3), C4' (PG9: 4 μ M, A549; 22 μ M, PC3), or C2' (PG13: 221 μ M, A549; 176 μ M, PC3) azido substitution afforded moderate to dramatic improvements in potency over the parent natural product (POH: 350 μ M, A549; 380 μ M, PC3). From this series, the C4'-azidosugar variant PG9 stands out as the best analogue, with >15 -fold improved potency against PC3 and >85 -fold increased potency against A549 in vitro, highlighting regioselectivity as an important contributor. Second, the benefits from sugar azido substitution are not additive, as evidenced by a comparison of PG2 (C6'-azido) and PG9 (C4'-azido) to the C4',C6'-diazido analogue PG20. Third, while the facile synthesis of the C6'-triazole-substituted analogue PG15 highlights the compatibility of neoglycosides with Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition chemistry, C6'-

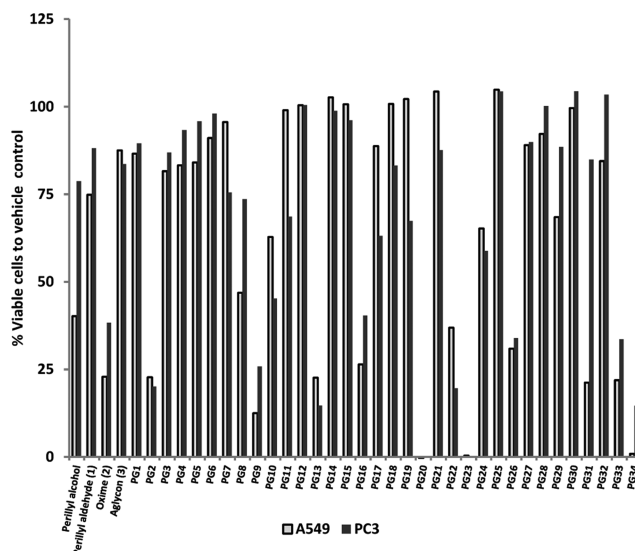


Figure 3. (a) Single dose (250 μ M) comparisons of POH neoglycosides and POH against A549 and PC3 cell lines.

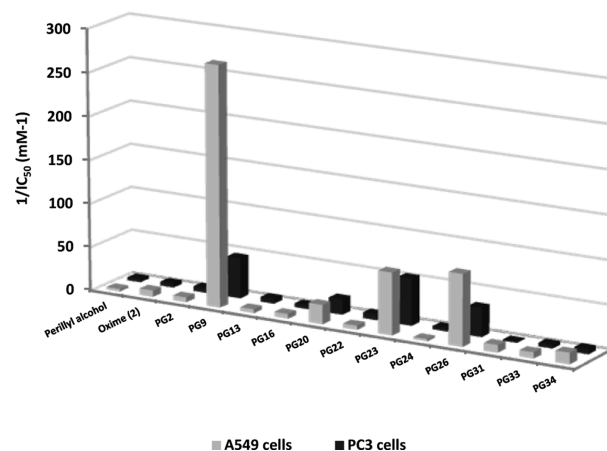


Figure 4. Reciprocal IC_{50} values for POH (IC_{50} 350 μ M, A549; 380 μ M, PC3) and the most active POH neoglycosides (the most active of which is PG9, with an IC_{50} 4 μ M, A549; 22 μ M, PC3). IC_{50} values and \pm SD are summarized in Supporting Information, Table S2 and represent a triplicate of dose-response experiments conducted over nine concentrations at 2-fold dilution.

triazole substitution is detrimental to activity based upon the comparative activities of PG2 (C6'-azido) and PG15. Fourth, sugar N-acylation also generally improved activity as both the 3'-N-decanoyl and 6'-N-decanoyl aminoglucosides (PG23 and PG26, respectively) displayed relatively similar improvements in potency over POH (12–20-fold, PC3; 20–25-fold, A549) with shorter C2', C3' and C6' N-acyl substitutions (e.g., PG16, PG27, PG31, PG33) generally being less favorable. Fifth, while the D-glucoside PG1 displayed activity similar to the parent POH (in a manner reminiscent to the prior work with 1a), removal of the C6'-CH₂OH (PG22) in this context led to slight improvements and, consistent with PG9, the addition of a 4'-azido-substitution (PG34: 76 μ M, A549; 170 μ M, PC3) led to further improvements in potency. Finally, on the basis of the comparative activities of PG9 and PG24, the D-enantiomer offered the greatest enhancement.

While a number of putative anticancer mechanisms for POH have been put forth, one reported effect centers around cap-

dependent translation in tumor cell lines. Specifically, Peffley and co-workers reported POH at 400 μM to suppress 4E-BP1 (Ser65/Thr37) phosphorylation and to disrupt interactions between critical components of the capped mRNA-binding complex (eIF4E and eIF4G).⁸ Thus, the effect of POH and representative glycosides **PG20** and **PG23** on 4E-BP1 phosphorylation was assessed using the cell line in which the most dramatic antiproliferative improvements were observed (A549). As illustrated in Figure 5, in A549 cells POH had no

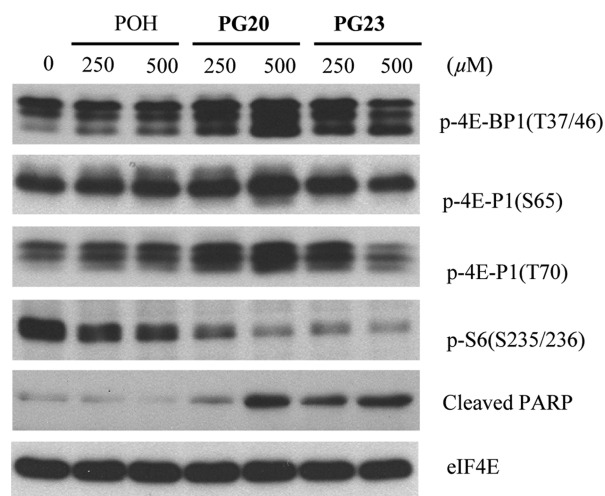


Figure 5. Western blot representing the effects of 250 and 500 μM perillyl alcohol (POH), **PG20**, and **PG23** treatments of A549 on phosphorylation of 4E-BP1 at 6 h.

effect upon the phosphorylation of 4E-BP1 at Thr37/46, Ser65, or Thr70, but 4E-BP1 phosphorylation was induced by **PG20** and **PG23**. Unlike POH, **PG20** and **PG23** also profoundly inhibited phosphorylation of S6 ribosomal protein, another important regulator of cap-dependent translation.⁵⁰ Furthermore, **PG20** and **PG23** substantially increased levels of cleaved PARP, an indicator of apoptosis, consistent with their increased antiproliferative activities. Cumulatively, these data suggest that the cytotoxicity of **PG20** and **PG23** toward A549 cells may be associated with the inhibition of phosphorylation of S6 ribosomal protein rather than 4E-BP1.

CONCLUSION

This study highlights a facile four-step process for POH glycodiversification and the discovery of a series of new POH glycosides that display improvements in potency approaching 2 orders of magnitude. For comparison, a survey of the previously reported activities of POH analogues (including glycosides, prodrug conjugates, and additional functionalized variations)^{21–27,51–54} revealed C7-*N,N*-disubstituted amino derivatives as among the most potent to date, the best of which displayed an $\text{IC}_{50} > 50 \mu\text{M}$ against A549. Thus, the activity of the POH 4'-azido-D-glucoside (**PG9**) reported herein stands out among most, if not all, POH derivatives reported to date. Cumulatively, the SAR presented suggests lipophilicity (azido⁵⁵ or *N*-acyl) to be of greatest benefit. While the fundamental antiproliferative mechanism of POH and POH congeners remains unclear, the current study also supports the contention that the newly reported POH glycosides may derive, at least in part, from the inhibition of phosphorylation of S6 ribosomal protein rather than 4E-BP1 as previously put forth.⁸ Additional

studies to specifically identify the target and assess the in vivo efficacy of these newly discovered POH glycosides are underway.

EXPERIMENTAL SECTION

All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. Sugar donors 2-deoxy-2-azido-D-glucose and 6-deoxy-6-azido-D-glucose were purchased from Carbo-synth (Berkshire, UK). Solvents were of ACS grade and purchased from Pharmco-AAPER (Brookfield, CT). TLC silica gel plates (60 F254) were purchased from EMD Chemicals Inc. (Gibbstown, NJ). NMR spectra were obtained on either a Varian Unity Inova 400 or 500 MHz instrument (Palo Alto, CA) at ambient temperature using 99.8% CDCl_3 and 99.8% CD_3OD (Cambridge Isotope Laboratories, MA, USA). ^1H and ^{13}C chemical shifts were referenced to internal solvent resonances and reported in parts per million (ppm), with coupling constants *J* given in Hz. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), dt (doublet of triplet), m (multiplet), and br (broad). HR-ESI-MS spectra were recorded on AB SCIEX Triple TOF 5600 system (AB Sciex, Framingham, MA, USA). Flash column chromatography was performed with RediSep Rf Gold columns from Teledyne Isco (Lincoln, NE) on a Biotage Isolera 4 (Biotage, Charlotte, NC, USA). Library member purity was assessed by ^1H NMR. The purity of starting material, (neoglycon **3**) and all corresponding neoglycosides was determined to be $\geq 95\%$ unless specified otherwise (Supporting Information, Table S1).

[(4S)-4-(prop-1-en-2-yl)cyclohex-1-en-1-yl]methanol (1). A suspension of POH (1 g, 6.6 mmol), IBX (45 wt %, 3.7 g, 2.0 mmol), and acetic acid (0.75 mL, 2.0 mmol) in acetonitrile (5 mL) was stirred vigorously at room temperature overnight. After completion of the reaction based upon TLC, sodium bicarbonate (200 mg) was added. The resulting mixture was filtered and the solvent was removed in vacuo to afford the crude product, which was purified by normal-phase flash chromatography using *n*-hexane/ethyl acetate as eluent (0.9 g, 88% yield). ^1H NMR (500 MHz, CDCl_3) δ 9.41 (s, 1H), 6.81–6.79 (m, 1H), 4.73 (d, 2H), 2.49–2.39 (m, 2H), 2.25–2.17 (m, 2H), 2.13–2.06 (m, 1H), 1.90–1.86 (m, 1H), 1.74 (s, 3H), 1.45–1.39 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 194.0, 150.7, 148.4, 141.4, 109.6, 40.8, 31.8, 26.5, 21.7, 20.8. HRMS-ESI (*m/z*): calcd for $\text{C}_{11}\text{H}_{18}\text{NO}$ (MH^+) 180.1383, found 180.1385.

Methoxy([(4S)-4-(prop-1-en-2-yl)cyclohex-1-en-1-yl]methylidene)amine (2). Perillyl aldehyde **1** (2 g, 13.3 mmol) was dissolved in absolute EtOH (40 mL). Et_3N (2.4 mL, 17.3 mmol) and methoxylamine hydrochloride (1.4 g, 17.3 mmol) were added, and the reaction was stirred overnight at room temperature. The solvent was removed in vacuo, and the crude product was purified by normal-phase flash chromatography using *n*-hexane/ethyl acetate as eluent (2.0 g, 84% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.59 (s, 1H), 5.93 (t, 1H), 4.70 (d, 2H), 3.80 (s, 3H), 2.43 (dd, 1H), 2.29–2.11 (m, 3H), 2.09–2.01 (m, 1H), 1.86–1.80 (m, 1H), 1.70 (s, 3H), 1.48–1.38 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 151.5, 149.1, 134.8, 132.7, 109.1, 61.6, 41.0, 31.4, 26.9, 24.0, 20.8. HRMS-ESI (*m/z*): calcd for $\text{C}_{11}\text{H}_{18}\text{NO}$ (MH^+) 180.1383, found 180.1385.

Methoxy([(4S)-4-(prop-1-en-2-yl)cyclohex-1-en-1-yl]methyl)amine (3). Oxime **2** (2.0 g, 11.2 mmol) was dissolved in MeOH (50 mL) under argon followed by the addition of NaNBH_3 (7.0 g, 111.6 mmol) and HOAc (3.2 mL, 55.8 mmol). The reaction was stirred at room temperature for 48 h, subsequently quenched with saturated aqueous NaHCO_3 (45 mL), and MeOH was removed in vacuo. The aqueous layer was extracted with CHCl_3 (40 mL \times 3). The recovered organics were washed with water and brine, dried over Na_2SO_4 , and subsequently filtered and concentrated in vacuo to provide crude product which was purified by normal-phase flash chromatography using *n*-hexane/ethyl acetate as eluent (1.0 g, 50% yield). ^1H NMR (500 MHz, CDCl_3) δ 5.61 (s, 1H), 5.52 (s, 1H), 4.67 (s, 2H), 3.48 (s, 3H), 3.37 (s, 2H), 2.19–2.02 (m, 4H), 1.98–1.85 (m, 1H), 1.84–1.74 (m, 1H), 1.69 (s, 3H), 1.48–1.38 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 149.9, 134.0, 124.4, 108.7, 61.8, 58.1, 41.1, 30.7,

27.7, 27.7, 20.9. HRMS-ESI (m/z): calcd for $C_{11}H_{20}NO$ (MH^+) 182.1539, found 180.1542.

General Neoglycosylation Procedure (PG1–PG6, PG8, PG9, PG12–PG14, PG16–PG18, PG20–PG34). Neoaglycon (**3**) was dissolved in MeOH/AcOH (5:1) in 1 dram vials along with stir fleas at a final concentration of 300–350 mM. Reducing sugar (2 equiv) was added and the vial capped, and the reaction was stirred at 40 °C for 24–48 h. Reaction progress was monitored by TLC. Upon completion, solvent was removed in vacuo to afford the crude product, which was purified by normal-phase flash chromatography using $CHCl_3$ /MeOH as eluent. The perillyl neoglycosides were obtained with an isolated yield ranging from 23% to 77% from perillyl neoaglycon **3** (Supporting Information, Table S1). Anomeric ratios were obtained by comparison of anomeric proton integration (Supporting Information, Table S1).

General Procedure for Azide Reduction (PG7, PG10, PG11, PG19). To a 100 mM solution of azidosugar glycoside in THF was added PMe_3 in THF (1.2 equiv), and the reaction was stirred at 50 °C for 1 h. After removal of the solvent in vacuo, the crude residue was purified by normal-phase column chromatography using $CHCl_3$ /MeOH as eluent to afford the desired aminosugar glycoside. The aminosugar-appended perillyl neoglycosides were obtained with an isolated yield ranging from 29% to 46% (Supporting Information, Table S1).

Cancer Cell Line Cytotoxicity Assay. Human cancer cell line (lung adenocarcinoma A549 and prostate cancer PC3) viability was determined using the alamar blue (or resazurine reduction) assay as previously described.^{33,56–58} Briefly, cells were seeded in F-12K medium (Kaighn's Modification of Ham's F-12 medium, Invitrogen), supplemented with 10% FBS, 2 mM L-glutamine, 100 μ g/mL penicillin, and 100 μ g/mL streptomycin onto flat-bottomed 96-well tissue culture plates (Corning, NY, USA) at a density of 3×10^3 cells/well and allowed to adhere overnight. After removing the medium, 100 μ L of fresh medium containing nine 2- or 3-fold dilutions (500 to 75 nM) of each compound were added. All assays were conducted in the presence of both negative (0.5% DMSO vehicle control) and positive (1.5 mM H_2O_2) controls. The plates were incubated for 48 h under standard conditions, 5% CO_2 and 37 °C, in a humidity control incubator. At the end of incubation period, 10 μ L of resazurin (0.25 mg/mL in water) was added to each well. Plates were incubated for an additional 3 h at standard culture conditions and shaken for 5–10 s, and fluorescence of resorufin (λ_{ex} = 600 nm, λ_{em} = 590 nm) was recorded as a basis for IC_{50} determination using a nonlinear interpolation of dose-dependent curves using Prism. Data presented (Figure 4 and Supporting Information, Table S2) represent mean values (\pm SD) of triplicate determinations from three independent experiments.

Immunoblot Analysis. A549 cells were treated with 250 and 500 μ M of perillyl alcohol (POH), PG20, and PG23 for 6 h. Cells were harvested, lysed, and the corresponding extracts subjected to SDS-PAGE, and analyzed by Western analysis as previously described.⁵⁹ Each SDS-PAGE sample contained equal amounts of total protein. Commercial primary antibodies for p-4E-BP1(T37/46), p-4E-BP1(S65), p-4E-BP1(T70), p-S6 (S235/236), eIF4E, and cleaved PARP (Cell Signaling Technology, Danvers, MA) were employed, and secondary antibodies were detected using chemiluminescence (GE Healthcare Bio-Sciences, Pittsburgh, PA).

■ ASSOCIATED CONTENT

■ Supporting Information

Synthetic procedure of sugars, characterization data of synthesized compounds, and activity results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): The authors report competing interests. JST is a co-founder of Centrose (Madison, WI).

■ ACKNOWLEDGMENTS

This work was supported by NIH R37 AI52218 (JST) and the National Center for Advancing Translational Sciences (UL1TR000117).

■ ABBREVIATIONS USED

POH, (S)-perillyl alcohol; TLC, thin layer chromatography; IC_{50} , concentration of agent that inhibits cell viability by 50%

■ REFERENCES

- (1) Kelloff, G. J.; Boone, C. W.; Crowell, J. A.; Steele, V. E.; Lubet, R. A.; Doody, L. A.; Malone, W. F.; Hawk, E. T.; Sigman, C. C. New agents for cancer chemoprevention. *J. Cell. Biochem.* **1996**, 26S, 1–28.
- (2) Simonsen, J. L.; Owen, L. N. *The Terpenes, Vol 1: The Simpler Acyclic and Monocyclic Terpenes and Their Derivatives*. Ed.; Cambridge University Press: London, 1947.
- (3) Belanger, J. T. Perillyl alcohol: applications in oncology. *Altern. Med. Rev.* **1998**, 3, 448–457.
- (4) Satomi, Y.; Miyamoto, S.; Gould, M. N. Induction of AP-1 activity by perillyl alcohol in breast cancer cells. *Carcinogenesis* **1999**, 20, 1957–1961.
- (5) Garcia, D. G.; Amorim, L. M. F.; Castro Faria, M. V.; de Freire, A. S.; Santelli, R. E.; Da Fonseca, C. O.; Quirico-Santos, T.; Burth, P. The anticancer drug perillyl alcohol is a Na/K-ATPase inhibitor. *Mol. Cell. Biochem.* **2010**, 345, 29–34.
- (6) Sundin, T.; Peffley, D. M.; Hentosh, P. Disruption of an hTERT–mTOR–RAPTOR protein complex by a phytochemical perillyl alcohol and rapamycin. *Mol. Cell. Biochem.* **2013**, 375, 97–104.
- (7) Peffley, D. M.; Gayen, A. K. Plant-derived monoterpenes suppress hamster kidney cell 3-hydroxy-3-methylglutaryl coenzyme A reductase synthesis at the transcriptional level. *J. Nutr.* **2003**, 133, 38–44.
- (8) Peffley, D. M.; Sharma, C.; Hentosh, P.; Buechler, R. D. Perillyl alcohol and genistein differentially regulate PKB/Akt and 4E-BP1 phosphorylation as well as eIF4E/eIF4G interactions in human tumor cells. *Arch. Biochem. Biophys.* **2007**, 465, 266–273.
- (9) Azzoli, C. G.; Miller, V. A.; Ng, K. K.; Krug, L. M.; Spriggs, D. R.; Tong, W. P.; Riedel, E. R.; Kris, M. G. A phase I trial of perillyl alcohol in patients with advanced solid tumors. *Cancer Chemother. Pharmacol.* **2003**, 51, 493–498.
- (10) Bailey, H. H.; Attia, S.; Love, R. R.; Fass, T.; Chappell, R.; Tutsch, K.; Harris, L.; Jumonville, A.; Hansen, R.; Shapiro, G. R.; Stewart, J. A. Phase II trial of daily oral perillyl alcohol (NSC 641066) in treatment-refractory metastatic breast cancer. *Cancer Chemother. Pharmacol.* **2008**, 62, 149–157.
- (11) Bailey, H. H.; Levy, D.; Harris, L. S.; Schink, J. C.; Foss, F.; Beatty, P.; Wadler, S. A phase II trial of daily perillyl alcohol in patients with advanced ovarian cancer: eastern cooperative oncology group study E2E96. *Gynecol. Oncol.* **2002**, 85, 464–468.
- (12) Crowell, P. L.; Siar Ayoubi, A.; Burke, Y. D. Antitumorogenic effects of limonene and perillyl alcohol against pancreatic and breast cancer. *Adv. Exp. Med. Biol.* **1996**, 401, 131–136.
- (13) Hudes, G. R.; Szarka, C. E.; Adams, A.; Ranganathan, S.; McCauley, R. A.; Weiner, L. M.; Langer, C. J.; Litwin, S.; Yeslow, G.; Halber, T.; Qian, M.; Gallo, J. M. Phase I pharmacokinetic trial of perillyl alcohol (NSC 641066) in patients with refractory solid malignancies. *Clin. Cancer. Res.* **2000**, 6, 3071–3080.

- (14) Liu, G.; Oettel, K.; Bailey, H.; Ummersen, L. V.; Tutsch, K.; Staab, M. J.; Horvath, D.; Alberti, D.; Arzoomanian, R.; Rezazadeh, H.; McGovern, J.; Robinson, E.; DeMets, D.; Wilding, G. Phase II trial of perillyl alcohol (NSC 641066) administered daily in patients with metastatic androgen independent prostate cancer. *Invest. New Drug* **2003**, *21*, 367–372.
- (15) Meadows, S. M.; Mulkerin, D.; Berlin, J.; Bailey, H.; Kolesar, J.; Warren, D.; Thomas, J. P. Phase II trial of perillyl alcohol in patients with metastatic colorectal cancer. *Int. J. Gastrointest. Cancer* **2002**, *32*, 125–128.
- (16) Ripple, G. H.; Gould, M. N.; Stewart, J. A.; Tutsch, K. D.; Arzoomanian, R. Z.; Alberti, D.; Feierabend, C.; Pomplun, M.; Wilding, G.; Bailey, H. H. Phase I clinical trial of perillyl alcohol administered daily. *Clin. Cancer Res.* **1998**, *4*, 1159–1164.
- (17) da Fonseca, C. O.; Simão, M.; Lins, I. R.; Caetano, R. O.; Futuro, D.; Quirico-Santos, T. Efficacy of monoterpene perillyl alcohol upon survival rate of patients with recurrent glioblastoma. *J. Cancer Res. Clin. Oncol.* **2011**, *137*, 287–293.
- (18) da Fonseca, C. O.; Schwartzmann, G.; Fischer, J.; Nagel, J.; Futuro, D.; Quirico-Santos, T.; Gattass, C. R. Preliminary results from a phase I/II study of perillyl alcohol intranasal administration in adults with recurrent malignant gliomas. *Surg. Neurol.* **2008**, *70*, 259–266.
- (19) Cho, H. Y.; Wang, W.; Jhaveri, N.; Torres, S.; Tseng, J.; Leong, M. N.; Lee, D. J.; Goldkorn, A.; Xu, T.; Petasis, N. A.; Louie, S. G.; Schöenthal, A. H.; Hofman, F. M.; Chen, T. C. Perillyl alcohol for the treatment of Temozolomide-resistant gliomas. *Mol. Cancer Ther.* **2012**, *11*, 2462–2472.
- (20) Gantt, R. W.; Peltier-Pain, P.; Thorson, J. S. Enzymatic methods for glyco(diversification/randomization) of drugs and small molecules. *Nat. Prod. Rep.* **2011**, *28*, 1811–1853.
- (21) Fujita, T.; Ohira, K.; Miyatake, K.; Nakano, Y.; Nakayama, M. Inhibitory effect of perilloside A and C, and related monoterpene glucosides on aldose reductase and their structure–activity relationships. *Chem. Pharm. Bull.* **1995**, *43*, 920–926.
- (22) Fujita, T.; Funayoshi, A.; Nakayama, M. A phenylpropanoid glucoside from *Perilla frutescens*. *Phytochemistry* **1994**, *37*, 543–546.
- (23) Fujita, T.; Nakayama, M. Monoterpene glucosides and other constituents from *Perilla frutescens*. *Phytochemistry* **1993**, *34*, 1545–1548.
- (24) Fujita, T.; Nakayama, M.; Perilloside, A. A monoterpene glucoside from *Perilla frutescens*. *Phytochemistry* **1992**, *31*, 3265–3267.
- (25) Xanthakis, E.; Magkouta, S.; Loutrari, H.; Stamatis, H.; Roussos, C.; Kolisis, F. N. Enzymatic synthesis of perillyl alcohol derivatives and investigation of their antiproliferative activity. *Biocatal. Biotransform.* **2009**, *27*, 1–9.
- (26) Shimoda, K.; Sakamoto, S.; Nakajima, N.; Hamada, H.; Hamada, H. Synthesis of unnatural mono- and oligosaccharides of farnesol, geraniol, and (S)-perillyl alcohol by biocatalytic glycosylations. *Chem. Lett.* **2008**, *37*, 556–557.
- (27) Arafat, H. M. M. Possible contribution of beta-glycosidases and caspases in the cytotoxicity of novel glycoconjugates in colon cancer cells. *Invest. New Drug* **2010**, *28*, 306–317.
- (28) Goff, R. D.; Thorson, J. S. Neoglycosylation and neoglycorandomization: enabling tools for the discovery of novel glycosylated bioactive probes and early stage leads. *MedChemComm* **2014**, *5*, 1036–1047, DOI: 10.1039/C4MD00117F.
- (29) Lin, C.-K.; Lu, T.-J. A simple method for the oxidation of primary alcohols with *o*-iodoxybenzoic acid (IBX) in the presence of acetic acid. *Tetrahedron* **2010**, *66*, 9688–9693.
- (30) Goff, R. D.; Thorson, J. S. Enhancement of cyclopamine via conjugation with nonmetabolic sugars. *Org. Lett.* **2012**, *14*, 2454–2457.
- (31) Peltier-Pain, P.; Timmons, S. C.; Grandemange, A.; Benoit, E.; Thorson, J. S. Warfarin glycosylation invokes a switch from anticoagulant to anticancer activity. *ChemMedChem* **2011**, *6*, 1347–1350.
- (32) Goff, R. D.; Thorson, J. S. Enhancing the divergent activities of betulinic acid via neoglycosylation. *Org. Lett.* **2009**, *11*, 461–464.
- (33) Zhang, J.; Ponomareva, L. V.; Marchillo, K.; Zhou, M.; Andes, D. R.; Thorson, J. S. Synthesis and antibacterial activity of doxycycline neoglycosides. *J. Nat. Prod.* **2013**, *76*, 1627–1636.
- (34) Griffith, B. R.; Krepel, C.; Fu, X.; Blanchard, S.; Ahmed, A.; Edmiston, C. E.; Thorson, J. S. A model for antibiotic optimization via neoglycosylation: synthesis of liponeoglycopeptides active against VRE. *J. Am. Chem. Soc.* **2007**, *129*, 8150–8155.
- (35) Kushwaha, D.; Dwivedi, P.; Kuanar, S. K.; Tiwari, V. K. Click reaction in carbohydrate chemistry: recent developments and future perspective. *Curr. Org. Synth.* **2013**, *10*, 90–135.
- (36) Fu, X.; Albermann, C.; Jiang, J. Q.; Liao, J. C.; Zhang, C. S.; Thorson, J. S. Antibiotic optimization via in vitro glycorandomization. *Nature Biotechnol.* **2003**, *21*, 1467–1469.
- (37) Carrasco, M. R.; Brown, R. T.; Serafimova, I. M.; Silva, O. Synthesis of N-Fmoc-O-(N'-Boc-N'-methyl)-aminohomoserine, an amino acid for the facile preparation of neoglycopeptides. *J. Org. Chem.* **2003**, *68*, 195–197.
- (38) Carrasco, M. R.; Brown, R. T. A versatile set of aminooxy amino acids for the synthesis of neoglycopeptides. *J. Org. Chem.* **2003**, *68*, 8853–8858.
- (39) Goff, R. D.; Thorson, J. S. Assessment of chemoselective neoglycosylation methods using chlorambucil as a model. *J. Med. Chem.* **2010**, *53*, 8129–8139.
- (40) Ahmed, A.; Peters, N. R.; Fitzgerald, M. K.; Watson, J. A., Jr.; Hoffmann, F. M.; Thorson, J. S. Colchicine glycorandomization influences cytotoxicity and mechanism of action. *J. Am. Chem. Soc.* **2006**, *128*, 14224–14225.
- (41) Goff, R. D.; Singh, S.; Thorson, J. S. Glycosyloxyamine neoglycosylation: a model study using calicheamicin. *ChemMedChem* **2011**, *6*, 774–776.
- (42) Peltier-Pain, P.; Marchillo, K.; Zhou, M. Q.; Andes, D. R.; Thorson, J. S. Natural product disaccharide engineering through tandem glycosyltransferase catalysis reversibility and neoglycosylation. *Org. Lett.* **2012**, *14*, 5086–5089.
- (43) Cipolla, L.; Peri, F. Carbohydrate-based bioactive compounds for medicinal chemistry applications. *Mini-Rev. Med. Chem.* **2011**, *11*, 39–54.
- (44) Langenhan, J. M.; Endo, M. M.; Engle, J. M.; Fukumoto, L. L.; Rogalsky, D. R.; Slevin, L. K.; Fay, L. R.; Lucker, R. W.; Rohlfing, J. R.; Smith, K. R.; Tjaden, A. E.; Werner, H. M. Synthesis and biological evaluation of RON-neoglycosides as tumor cytotoxins. *Carbohydr. Res.* **2011**, *346*, 2663–2676.
- (45) Langenhan, J. M.; Mullarky, E.; Rogalsky, D. K.; Rohlfing, J. R.; Tjaden, A. E.; Werner, H. M.; Rozal, L. M.; Loskot, S. A. Amphimediosides A–C: synthesis, chemoselective glycosylation, and biological evaluation. *J. Org. Chem.* **2013**, *78*, 1670–1676.
- (46) Langenhan, J. M.; Griffith, B. R.; Thorson, J. S. Neoglycorandomization and chemoenzymatic glycorandomization: two complementary tools for natural product diversification. *J. Nat. Prod.* **2005**, *68*, 1696–1711.
- (47) Langenhan, J. M.; Engle, J. M.; Slevin, L. K.; Fay, L. R.; Lucker, R. W.; Smith, K. R.; Endo, M. M. Modifying the glycosidic linkage in digitoxin analogs provides selective cytotoxins. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 670–673.
- (48) Carrasco, M. R.; Alvarado, C. I.; Dashner, S. T.; Wong, A. J.; Wong, M. A. Synthesis of aminooxy and N-alkylaminooxy amines for use in bioconjugation. *J. Org. Chem.* **2010**, *75*, 5757–5759.
- (49) Filira, F.; Biondi, B.; Biondi, L.; Giannini, E.; Gobbo, M.; Negri, L.; Rocchi, R. Opioid peptides: synthesis and biological properties of [(N'-glucosyl,N'-methoxy)- α,γ -diamino-(S)-butanoyl]⁴-deltorphin-1-neoglycopeptide and related analogues. *Org. Biomol. Chem.* **2003**, *1*, 3059–3063.
- (50) Ruvinsky, I.; Meyuhas, O. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. *Trends Biochem. Sci.* **2006**, *31*, 342–348.
- (51) Eumme, J. T.; Gibbs, B. S.; Zahn, T. J.; Sebolt-Leopold, J. S.; Gibbs, R. A. Novel limonene phosphonate and farnesyl diphosphate analogues: design, synthesis, and evaluation as potential protein-farnesyl transferase inhibitors. *Bioorg. Med. Chem.* **1999**, *7*, 241–250.

(52) Das, B. C.; Mahalingam, S. M.; Panda, L.; Wang, B.; Campbell, P. D.; Evans, T. Design and synthesis of potential new apoptosis agents: hybrid compounds containing perillyl alcohol and new constrained retinoids. *Tetrahedron Lett.* **2010**, *51*, 1462–1466.

(53) Chen, T.; Levin, D.; Pupalli, S. *Pharmaceutical compositions comprising POH derivatives*. WO2012027693, 2012.

(54) Hui, Z.; Zhang, M.; Cong, L.; Xia, M.; Dong, J. Synthesis and antiproliferative effects of amino-modified perillyl alcohol derivatives. *Molecules* **2014**, *19*, 6671–6682.

(55) Zimmerman, T. P.; Mahony, W. B.; Prus, K. L. 3'-Azido-3'-deoxythymidine. An unusual nucleoside analogue that permeates the membrane of human erythrocytes and lymphocytes by nonfacilitated diffusion. *J. Biol. Chem.* **1987**, *262*, 5748–5754.

(56) Wang, X. C.; Shaaban, K. A.; Elshahawi, S. I.; Ponomareva, L. V.; Sunkara, M.; Zhang, Y. A.; Copley, G. C.; Hower, J. C.; Morris, A. J.; Kharel, M. K.; Thorson, J. S. Frenolicins C–G, pyranonaphthoquinones from *Streptomyces* sp RM-4-15. *J. Nat. Prod.* **2013**, *76*, 1441–1447.

(57) Shaaban, K. A.; Wang, X. C.; Elshahawi, S. I.; Ponomareva, L. V.; Sunkara, M.; Copley, G. C.; Hower, J. C.; Morris, A. J.; Kharel, M. K.; Thorson, J. S. Herbimycins D–F, ansamycin analogues from *Streptomyces* sp. RM-7–15. *J. Nat. Prod.* **2013**, *76*, 1619–1626.

(58) Shaaban, K. A.; Singh, S.; Elshahawi, S. I.; Wang, X. C.; Ponomareva, L. V.; Sunkara, M.; Copley, G. C.; Hower, J. C.; Morris, A. J.; Kharel, M. K.; Thorson, J. S. Venturicidin C, a new 20-membered macrolide produced by *Streptomyces* sp TS-2–2. *J. Antibiot.* **2014**, *67*, 223–230.

(59) She, Q.-B.; Halilovic, E.; Ye, Q.; Zhen, W.; Shirasawa, S.; Sasazuki, T.; Solit, D. B.; Rosen, N. 4E-BP1 is a key effector of the oncogenic activation of the AKT and ERK signaling pathways that integrates their function in tumors. *Cancer Cell* **2010**, *18*, 39–51.