Tetrahedron 67 (2011) 10216–10221

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Design and synthesis of a potential SH2 domain inhibitor bearing a stereodiversified 1,4-*cis*-enediol scaffold

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ARTICLE INFO

Article history:
Received 26 September 2011
Received in revised form 3 October 2011
Accepted 4 October 2011
Available online 12 October 2011

Keywords:
cis-Enediol
SH2 domain
Stereodiversified
Alkynylation

ABSTRACT

Synthesis of a potential Src family SH2 domain inhibitor incorporating a 1,4-*cis*-enediol scaffold is reported. The synthetic route offers straightforward and highly selective access to the enediol and its associated chiral centers. Key steps include stereocontrolled *syn*-aldol coupling, amide alkynylation, and asymmetric ketone reduction.

1. Introduction

Protein tyrosine kinase (PTK) signal transduction pathways control a host of important cellular functions, including metabolism, growth and proliferation, and apoptosis.[1] As a result, aberrant PTK signaling often results in disease, most notably cancer and autoimmune diseases.[2] The Src homology-2 (SH2) domain, composed of ~100 amino acids, is a non-catalytic phosphotyrosine binding domain found in cytosolic PTKs which, by virtue of its central role in mediating PTK signaling, has emerged as an important target for therapeutic intervention.[3,4] Small peptides containing a phosphotyrosine (pTyr) can effectively inhibit SH2 domain binding of cognate proteins in the signal cascade, and therefore provide a logical starting point for inhibitor design.[5,6] Among PTKs of the Src family, pTyr-containing substrates generally adopt a similar binding mode, as exemplified by Lck kinase binding to tetrapeptide Ac-pTyr-Glu-Glu-Ile, or pYEEI peptide.[7] A highly conserved pocket binds the pTyr, and a second pocket three residues away (the 'pY+3' pocket) binds a hydrophobic substituent. The two pockets are connected by a narrow β-sheet region, which protrudes into the solvent. This 'plug-and-socket' binding mode can be used to derive a general model for inhibitor design (Fig. 1A).[4] Despite the extensive structural data available, efforts toward the development of new and therapeutically useful SH2 domain inhibitors have met with limited success, and progress remains slow.[4,8,9] One major challenge has been the intracellular penetration of a free phosphate group. This challenge has been addressed by various prodrug strategies, including a phosphoramidate-based prodrug developed in our laboratory.[10] Further issues to be addressed include reducing peptide character, and expanding ligand diversity in order to improve potency and selectivity. An additional consideration is the role of conformational restraint in ligand binding.[11] In summary, *an ideal inhibitor would be non-peptidic, allow for structural and stereochemical diversity, and provide a means to introduce conformational restraint.*

An enediol-based scaffold meets all of these criteria, and seemed to us an attractive choice for SH2 inhibitor design, especially given the report in the literature of a series of nanomolar μ-opioid receptor agonists based on a 1,5-enediol system.[12] We envisioned an inhibitor model that would incorporate a 1,4-*cis*-enediol core, a phosphotyrosine mimic, and a hydrophobic substituent. As the linking segment, the *cis*-alkene would provide a degree of conformational restraint and presumably serve to orient the ligands into their respective pockets by wrapping around the central β-sheet region. The associated chiral centers would allow for the systematic comparison of stereochemical preferences within the SH2 domain. A benzyl group was chosen as the pY+3 substituent based on previous reports in the literature highlighting its suitability.[13,14] The stereoisomer exhibiting the greatest binding affinity will ultimately be synthesized as its corresponding prodrug[10] for cell-based and in vivo studies (Fig. 2).

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doi:10.1016/j.tet.2011.10.014

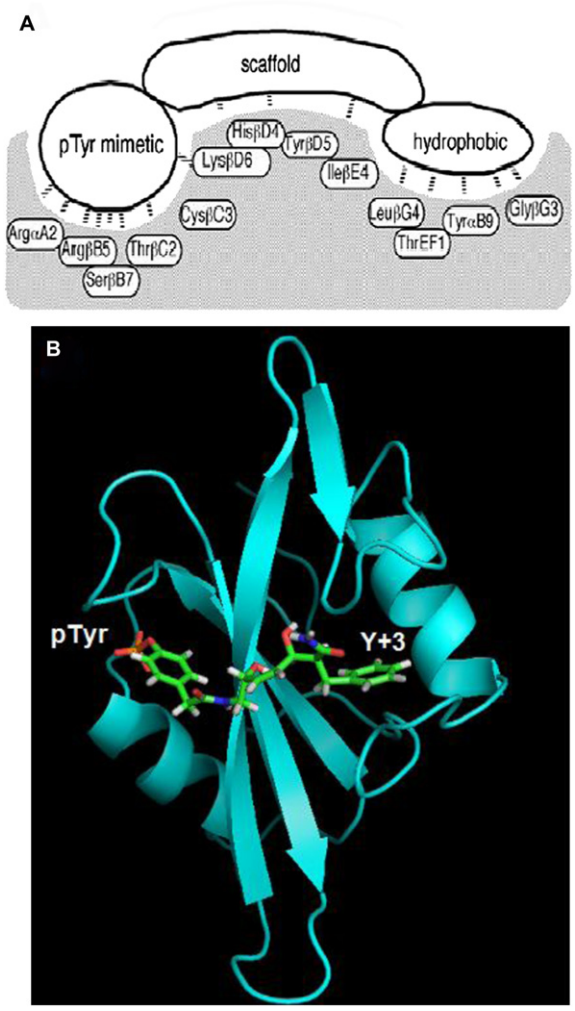


Fig. 1. A. General ligand binding mode for Src family SH2 domains. Figure adapted from Machida and Mayer.[4] B. Ribbon diagram of *cis*-enediol inhibitor docked to Lck SH2 domain (PDB 1Lkk).

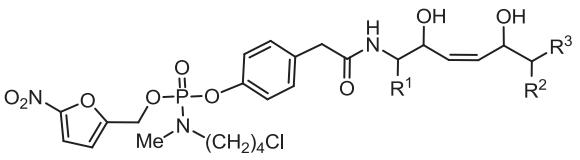
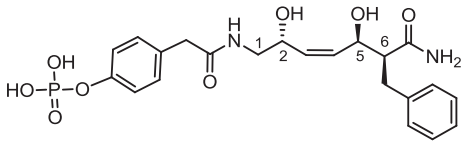


Fig. 2. *cis*-Enediol phosphoramidate prodrug.

2. Results and discussion

Computational docking of proposed inhibitors to the Lck SH2 domain (PDB 1Lkk) supported our reasoning and generated structures similar to pYEEI-bound Lck (Fig. 1B). Substituents at C-1 contributed little to binding, so they were not considered further.

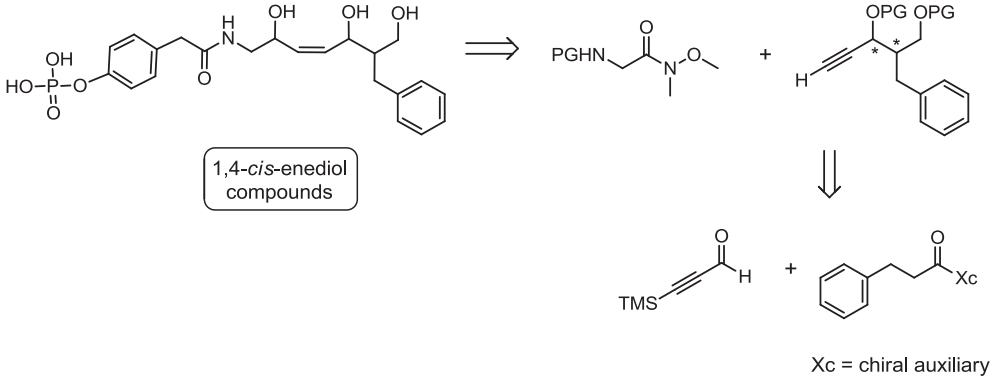
A preliminary synthesis of compound **2** was carried out, using a published procedure to access the *cis*-enediol core via Grubbs ring-closing metathesis.[15] We found the synthesis to be laborious and inefficient, but testing of **2** in a competitive fluorescence binding assay gave an IC_{50} comparable to that of pYEEI peptide (Huang and Borch, unpublished). These results suggested that the *cis*-enediol scaffold could be used to generate high-affinity SH2 domain ligands, and encouraged us to develop an improved synthetic pathway to the proposed compounds.



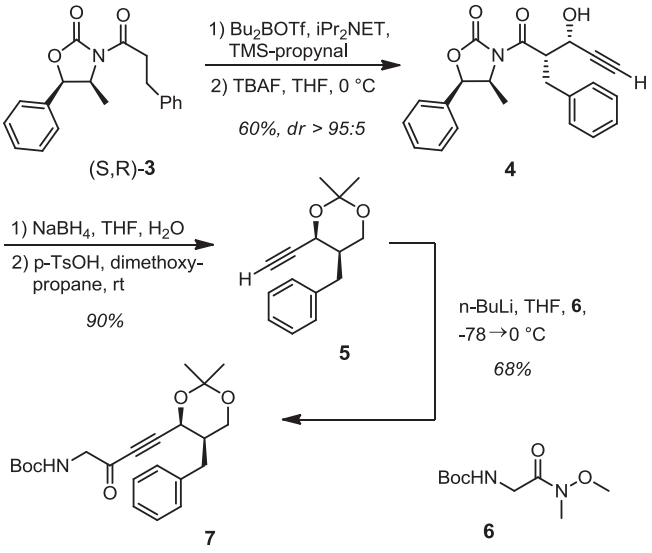
2

Our new synthetic approach is focused on efficient conversions and installation of the three chiral centers with high selectivity. In addition, the synthesis allows for further modification of the linker region, which may be of use in future studies. Computational modeling studies showed the terminal amide of **2** directed outward into the solvent rather than interacting with the protein, so it was replaced with a hydroxyl group. The pathway to all eight stereoisomers is outlined in Scheme 1. 1,4-*cis*-Enediol compounds **1** can be made by coupling a protected chiral alkyne and Weinreb amide. The alkyne can in turn be synthesized by Evans *syn*[16] or Masamune *anti*[17] stereocontrolled aldol coupling of TMS-propynal with the appropriate homobenzyl substituted chiral auxiliary. Herein we describe the synthesis of the (*R*,*R*,*R*)-stereoisomer.

Synthesis of ynone **7** is shown in Scheme 2. Introduction of the chiral centers at carbons 5 and 6 was achieved by Evans asymmetric *syn*-aldol addition of trimethylsilylpropynal to chiral auxiliary (*S*,*R*)-**3**.[18] Subsequent silyl cleavage with TBAF yielded aldol product **4**, which was isolated with a diastereomeric ratio of 96:4 after column chromatography. Reductive cleavage of the chiral auxiliary with sodium borohydride and acetonide protection of the resulting diol afforded alkyne **5**. The lithium alkynylide was then added to Weinreb amide **6** to give ynone **7** in 68% yield. An alkyne to amide ratio of 2:1 was necessary to obtain reasonable yields for this step, but unreacted alkyne was easily recovered. Zinc-mediated addition of the alkyne to Boc-glycinal was also tried under numerous conditions, but did not yield any product.

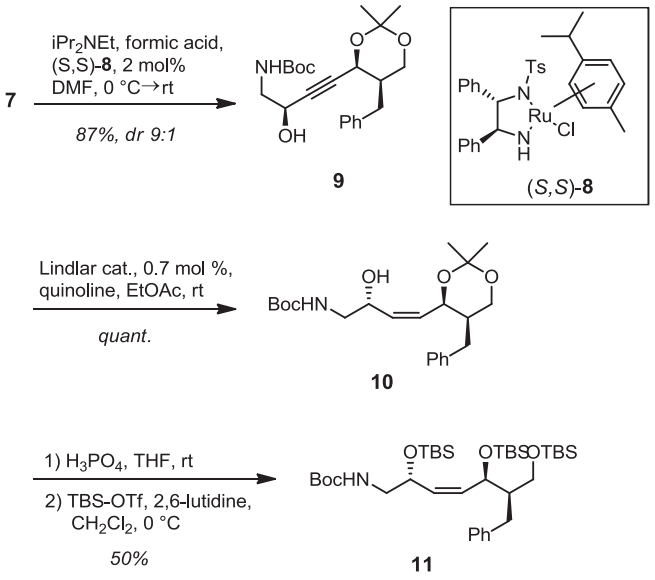


Scheme 1. Retrosynthetic analysis of modified 1,4-*cis*-enediol compounds (PG=protecting group).



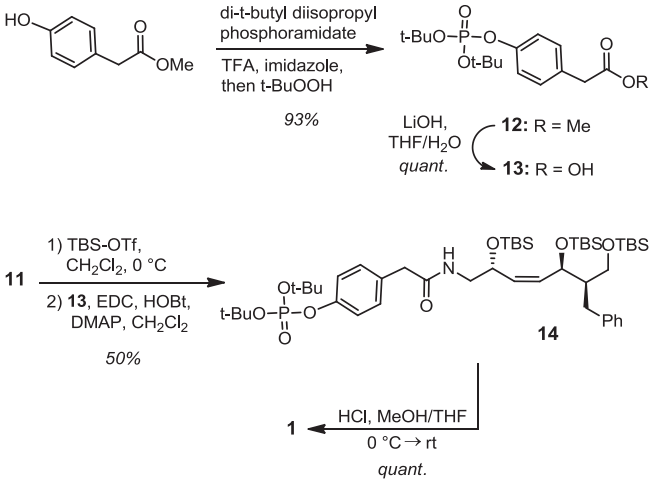
Scheme 2. Preparation of ynone **7**.

The next step required asymmetric reduction of ynone **7** to give the desired propargyl alcohol (Scheme 3). Reduction with methyl-CBS-oxazaborolidine gave poor selectivity, but asymmetric transfer hydrogenation with Noyori's chiral ruthenium catalyst[19] installed the third and final chiral center at carbon 2 with high yield and selectivity (dr 9:1). Stereochemical assignment was determined by Mosher analysis of the *O*-MTPA esters.[20] Propargyl alcohol **9** was then reduced to *cis*-alkene **10** via hydrogenation with Lindlar catalyst. At this point a protecting group exchange became necessary to ensure selective removal of the Boc group in the next step, so the 1,3-acetonide was cleaved and the resulting triol was protected by introduction of TBS groups to give protected aminotriol **11**.



Scheme 3. Synthesis of protected aminotriol **11**.

Completion of (*R*,*R*,*R*)-**1** is detailed in Scheme 4. *tert*-Butyl phosphoryl acid **13** was synthesized in two steps from commercially available methyl 4-hydroxyphenylacetate.[21] Boc cleavage of **11** with TBS-triflate followed by coupling to acid **13** with EDC/HOBt afforded acylation product **14** in good yield (Scheme 4). Global deprotection under acidic conditions completed the synthesis and gave (*R*,*R*,*R*)-**1** in essentially quantitative yield.



Scheme 4. Completion of (*R*,*R*,*R*)-**1**.

3. Conclusion

In conclusion, we have synthesized a novel Src family SH2 domain inhibitor based on a 1,4-*cis*-enediol core. Our synthesis offers straightforward and highly selective access to a privileged molecular scaffold, and will allow the study of stereochemical preferences within the SH2 domain. Synthesis of the remaining seven diastereomers is underway, and binding data for the entire series will be reported shortly.

4. Experimental

4.1. General procedures

All NMR spectra were recorded on a Bruker Avance DPX-300 spectrometer equipped with a multinuclear (^{31}P, ^{13}C, ^{1}H, and ^{19}F) probe. Chemical shifts are given in parts per million with reference to tetramethylsilane. All ^{31}P NMR spectra were acquired using broadband gated decoupling. ^{31}P chemical shifts are reported in parts per million using 1% triphenylphosphine oxide in benzene-d_6 as the coaxial reference set at 0. Mass spectral data were obtained from the Purdue University Mass Spectrometry Service, West Lafayette, IN. IR spectra were recorded on a Perkin Elmer Spectrum I FTIR spectrometer using a NaCl plate. Optical rotations were measured on a Perkin Elmer 341 polarimeter with 0.5 dm path length cell. Analytical HPLC analysis was done using a Beckman System Gold equipped with a 168 detector set at 215 nm and a Zorbax Eclipse 5-μ analytical C18 column (4.6×250 mm) from Agilent Technologies Inc. Lck GST-SH2 protein was a kind gift from Dr. Robert Geahlen. All moisture-sensitive reactions were carried out under an atmosphere of argon, and all organic solvents were distilled prior to use unless otherwise specified. Flash chromatography using silica gel grade 60 (230–400 mesh) was carried out for all chromatographic separations. Thin layer chromatography was performed using Analtech glass plates precoated with silica gel (250 nm), and visualized using UV or phosphomolybdic acid stain. All reagents were purchased from Aldrich, Alfa-Aesar, or TCI America, unless stated otherwise.

4.2. Chemistry

*4.2.1. (4S,5R)-3-((2S,3S)-2-Benzyl-3-hydroxypent-4-ynoyl)-4-methyl-5-phenyloxazolidin-2-one (**4**).* (*S*,*R*)-**3** (6.56 g, 21.2 mmol) was dissolved in 100 mL dry CH_2Cl_2 and cooled to 0 °C. Dibutylboron triflate (23 mL, 1.0 M in CH_2Cl_2, 23.3 mmol) was added

dropwise, and the light orange reaction mixture allowed to stir for 10 min at 0 °C. Diisopropylethylamine (4.42 mL, 25.4 mmol) was then added dropwise, and the resulting clear solution allowed to stir for 30 min at 0 °C. The mixture was then cooled to −78 °C, and a solution of trimethylsilylpropynal (3.1 mL, 21.0 mmol) in 30 mL CH_2Cl_2 at −78 °C was added via cannula. After 20 min stirring at −78 °C, the reaction mixture was warmed to 0 °C and allowed to stir for an additional 2 h at that temperature, then quenched by dropwise addition of the following, in the order listed: 25 mL phosphate buffer (pH 7.4), 65 mL methanol, and 65 mL of a 2:1 solution of methanol and 30% aqueous H_2O_2, cooled to 0 °C. The mixture was allowed to stir at 0 °C for 1 h, then extracted 3× with CH_2Cl_2. The combined organic layers were washed with $NaHCO_3$ and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was dissolved in 135 mL THF and 9 mL MeOH and cooled to −20 °C. Tetrabutylammonium fluoride (21 mL, 1.0 M in THF, 21.2 mmol) was added dropwise, and the mixture allowed to stir for 2 h at 0 °C. The reaction was quenched with satd NH_4Cl solution and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over anhydrous Na_2SO_4, followed by removal of solvent under reduced pressure. The crude product was purified by column chromatography (20% ethyl acetate/hexane) to yield 5.8 g (60% over two steps) of major diastereomer (*S*,*R*)-**4** as an off-white foam. TLC 50% ethyl acetate/hexane R_f=0.7 visualized with PMA or UV. Diastereomeric ratio after flash column chromatography was 96:4, as determined by reverse phase HPLC. HPLC, Zorbax XDB-C18 (250×4.6 mm, 5μ); flow=1.0 mL/min; λ=215 nm; isocratic (80:20 MeCN/ddH_2O); Major isomer t_R=7.15 min. $[\alpha]_D^{25}$ −34.0 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.16–7.39 (m, 10H), 5.60 (d, *J*=7.4 Hz, 1H), 4.66–4.80 (m, 2H), 4.64 (s, 1H), 3.18 (d, *J*=6.4 Hz, 2H), 2.71 (br, 1H), 2.62 (d, 1H), 0.60 (d, *J*=6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.6, 152.8, 137.6, 133.0, 129.3, 128.7, 128.6, 128.3, 126.6, 125.6, 81.8, 78.7, 77.2, 75.0, 63.1, 54.7, 49.8, 34.5, 14.1. ESI (+) HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{22}H_{21}NO_4$ 386.1368; found, 386.1370.

*4.2.2. (4S,5R)-5-Benzyl-4-ethynyl-2,2-dimethyl-1,3-dioxane (**5**).* Aldol product **4** (415 mg, 1.14 mmol) was dissolved in 30 mL THF and cooled to 0 °C. $NaBH_4$ (173 mg, 4.56 mmol) was dissolved in 7 mL H_2O and added dropwise to the THF solution. The mixture was allowed to warm to room temperature and stirred for 1 h, after which it was cooled to 0 °C and quenched with NH_4Cl. The mixture was again brought to room temperature and stirred for 1 h. The layers were separated, and the aqueous layer extracted 3× with EtOAc. The combined organic layer was washed with $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The chiral auxiliary was recovered by recrystallization of the crude residue with ethyl acetate/hexanes (~1:1). The remaining oil was then dissolved in 23 mL 2,2-dimethoxypropane. *p*-Toluenesulfonic acid monohydrate (22 mg, 0.114 mmol) was added in one portion, and the mixture stirred for 1 h at room temperature. The mixture was then diluted with CH_2Cl_2 and quenched with $NaHCO_3$ at 0 °C. The layers were separated and the aqueous layer extracted 3× with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield 236 mg pure alkyne **5** as a light yellow oil (90% over two steps). TLC 30% ethyl acetate/hexane R_f=0.9 visualized with PMA. $[\alpha]_D^{25}$ +22.6 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.21–7.34 (m, 5H), 4.92 (t, 1H), 3.80 (dd, *J*=2.8, 12.1 Hz, 1H), 3.65 (dd, *J*=3.3, 12.0 Hz, 1H), 2.99–3.11 (m, 2H), 2.62 (d, *J*=2.4 Hz, 1H), 1.82–1.87 (m, 1H), 1.57 (s, 3H), 1.47 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 140.0, 129.3, 128.4, 126.1, 99.4, 81.4, 75.2, 64.2, 60.8, 39.6, 31.6, 28.4, 20.8. CI (+) LRMS (*m*/*z*): 231 $[M+H]^+$.

*4.2.3. tert-Butyl 2-(methoxy(methyl)amino)-2-oxoethylcarbamate (**6**).* Weinreb amide **6** was synthesized from Boc-glycine as described by Morwick et al.[22] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.29 (br, 1H), 4.01 (d, *J*=4.0 Hz, 2H), 3.65 (s, 3H), 3.14 (s, 3H), 1.38 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.1, 155.8, 79.5, 61.3, 41.6, 32.2, 28.2. CI (+) LRMS (*m*/*z*): 219 $[M+H]^+$.

*4.2.4. tert-Butyl-4-((4S,5R)-5-benzyl-2,2-dimethyl-1,3-dioxan-4-yl)-2-oxobut-3-ynylcarbamate (**7**).* Alkyne **5** (3.27 g, 14.2 mmol) was dissolved in 68 mL dry THF and cooled to −78 °C. *n*-BuLi (8.37 mL, 1.78 M in hexane, 14.9 mmol) was added dropwise and the resulting mixture stirred at −78 °C for 1 h. The mixture was then added via cannula to a solution of Weinreb amide **2** (1.55 g, 7.10 mmol) in 35 mmol THF cooled to −78 °C. The mixture was then stirred at 0 °C for 3 h, cooled to −78 °C, and quenched by slow addition of satd NH_4Cl solution. The resulting mixture was extracted 3× with Et_2O, and the combined organic layers washed with $NaHCO_3$ and brine and dried over anhydrous Na_2SO_4. Purification of the crude oil by column chromatography (10%→20% ethyl acetate/hexane) yielded 1.87 g ynone **7** as a yellow oil (68%). Starting alkyne (1.33 g (82%)) was also recovered. TLC 30% ethyl acetate/hexane R_f=0.5 visualized with PMA. $[\alpha]_D^{25}$ +34.0 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.16–7.32 (m, 5H), 5.20 (br, 1H), 5.02 (d, *J*=3.4 Hz, 1H), 4.15 (d, *J*=5.3 Hz, 2H), 3.82 (dd, *J*=3.0, 12.1 Hz, 1H), 3.64 (dd, *J*=4.0, 12.1 Hz, 1H), 2.91–2.98 (m, 2H), 1.96–2.00 (m, 1H), 1.53 (s, 3H), 1.44 (s, 12H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 182.5, 155.4, 139.0, 129.2, 128.5, 126.3, 99.7, 91.9, 83.4, 80.2, 64.3, 60.9, 52.1, 39.1, 32.2, 28.2, 27.7, 21.5. ESI (+) HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{22}H_{29}NO_5$ 410.1943; found, 410.1946.

*4.2.5. tert-Butyl-((R)-4-((4S,5R)-5-benzyl-2,2-dimethyl-1,3-dioxan-4-yl)-2-hydroxybut-3-yn-1-yl)carbamate (**9**).* To a stirred solution of ynone **7** (480 mg, 1.24 mmol) in 9.5 mL anhydrous dimethylformamide under argon atmosphere was added (*S*,*S*)-Noyori catalyst **8** (16 mg, 0.025 mmol) at room temperature. In a separate flask, diisopropylethylamine (208 mg, 1.61 mmol) was cooled to −10 °C, and 98% formic acid (188 mg, 4.09 mmol) was added dropwise, keeping the temperature below 0 °C. This solution was added dropwise to the ynone, and the reaction mixture allowed to stir overnight at room temperature. The mixture was then cooled to 0 °C and quenched by addition of satd $NaHCO_3$, extracted 3× with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by column chromatography (10%→50% ethyl acetate/hexane) to give 420 mg of propargyl alcohol **9** as a light yellow oil (87%, dr 9:1). Stereochemical assignment of the alcohol configuration was determined by Mosher analysis of the (*R*) and (*S*)-MTPA esters.[16] Diastereomeric ratio was determined by ^{19}F NMR of the MTPA esters. TLC 30% ethyl acetate/hexane R_f=0.4 visualized with PMA. $[\alpha]_D^{23}$ +22.0 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.15–7.29 (m, 5H), 5.21 (t, *J*=5.5 Hz, 1H), 4.91 (s, 1H), 4.54 (br, 1H), 3.78 (dd, *J*=2.6, 11.8 Hz, 1H), 3.59 (dd, *J*=3.1, 12.0 Hz, 1H), 3.30–3.46 (m, 3H), 2.90–3.04 (m, 2H), 1.80 (m, 1H), 1.50 (s, 3H), 1.42 (s, 3H), 1.41 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.6, 139.9, 129.3, 128.3, 126.0, 99.4, 85.7, 83.0, 79.8, 77.3, 64.3, 62.0, 60.9, 46.5, 39.6, 31.8, 28.4, 20.7. ESI (+) HRMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{22}H_{31}NO_5$, 412.2100; found, 412.2093.

*4.2.6. tert-Butyl-((R,Z)-4-((4R,5R)-5-benzyl-2,2-dimethyl-1,3-dioxan-4-yl)-2-hydroxybut-3-en-1-yl)carbamate (**10**).* Propargyl alcohol **9** (271 mg, 0.693 mmol) was dissolved in 8.5 mL ethyl acetate and flushed with argon. Lindlar catalyst (5% palladium on calcium carbonate, poisoned with lead; 52 mg, 0.485 mmol) and quinoline (6 μL, 0.049 mmol) were added. The flask was flushed with argon again, and then flushed with H_2 gas 3×. A hydrogen balloon was then added, and the mixture allowed to stir overnight at room temperature. The following day, the reaction mixture was filtered through Celite®, rinsing with ethyl acetate. The organic filtrate was

washed with 1 M HCl, satd $NaHCO_3$, and brine, then dried over anhydrous Na_2SO_4 and concentrated to yield 273 mg alkene **10** as a light brown foam (quantitative). TLC 30% ethyl acetate/hexane R_f=0.4 visualized with PMA. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.14–7.28 (m, 5H), 5.59–5.62 (m, 2H), 5.15 (br, 1H), 4.97 (br, 1H), 4.54 (q, J=5.3 Hz, 1H), 3.85 (d, J=11.6 Hz, 1H), 3.53 (d, J=11.9 Hz, 1H), 3.20–3.32 (m, 3H), 2.77–3.02 (m, 2H), 1.66 (d, J=11.6 Hz, 1H), 1.49 (s, 6H), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.6, 140.5, 132.2, 131.3, 129.3, 128.3, 125.9, 99.0, 79.5, 70.0, 67.9, 61.6, 46.4, 39.9, 30.2, 29.7, 28.3, 19.1. ESI (+) HRMS (m/z): $[M+Na]^+$ calcd for $C_{22}H_{33}NO_5$, 414.2256; found, 414.2273.

*4.2.7. tert-Butyl-((2R,5R,6R,Z)-6-benzyl-2,5,7-tris((tert-butyldimethylsilyl)oxy)hept-3-en-1-yl)carbamate (**11**).* Alkene **10** (216 mg, 0.552 mmol) was dissolved in 3.5 mL THF and cooled to 0 °C. Phosphoric acid of 85% (566 μL, 8.28 mmol) was added dropwise, and the resulting solution stirred at room temperature for 3 h. The mixture was then cooled back down to 0 °C and diluted with H_2O. Aq NaOH of 50 wt % was added dropwise to bring the solution to pH 8, and the solution was poured into brine and extracted 3× with EtOAc, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude oil was then dissolved in 9 mL anhydrous CH_2Cl_2 and cooled to −78 °C. 2,6-Lutidine (322 μL, 2.76 mmol) was added dropwise, followed by TBS-trifluoromethanesulfonate (507 μL, 2.21 mmol). The reaction mixture was stirred overnight at room temperature, after which it was quenched with MeOH at 0 °C. The mixture was then poured into brine and extracted 3× with CH_2Cl_2, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude oil was purified by column chromatography (3% ether/pentane) to yield 190 mg pure tris-TBS aminotriol **11** as a clear, colorless oil (50% over two steps). TLC 10% ethyl acetate/hexane R_f=0.7 visualized with PMA. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.12–7.26 (m, 5 Hz), 5.50–5.57 (m, 1H), 5.19–5.26 (m, 1H), 4.80–4.83 (d, J=8.4 Hz, 1H), 4.66 (br, 1H), 4.46–4.52 (m, 1H), 3.09–3.51 (m, 4H), 2.80 (dd, J=4.6, 14.1 Hz, 1H), 2.43 (dd, J=9.8, 14.0 Hz, 1H), 1.92 (br, 1H), 1.44 (s, 9H), 0.85–0.92 (m, 27H), −0.06–0.09 (m, 18H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.6, 141.1, 134.9, 129.6, 128.9, 128.1, 125.6, 78.8, 68.1, 66.7, 61.7, 50.5, 46.4, 31.1, 28.3, 25.9, 18.1, −4.0, −4.1, −4.6, −4.9, −5.6. ESI (+) LRMS (m/z): 381 $[M+Na]^+$.

*4.2.8. Methyl-2-(4-((di-tert-butoxyphosphoryl)-oxy)phenyl)acetate (**12**).* To a stirred solution of imidazole (188 mg, 2.76 mmol) in 750 μL anhydrous THF at room temperature under argon atmosphere was added trifluoroacetic acid (210 μL, 2.76 mmol) dropwise. The resulting slurry was stirred at room temperature for 10 min, after which di-*tert*-butyl *N*,*N*-diisopropylphosphoramidite (285 μL, 0.90 mmol) was added dropwise. After stirring for an additional 10 min, a solution of methyl 4-hydroxyphenylacetate (100 mg, 0.60 mmol) in 300 μL anhydrous THF was added dropwise over 15 min. The solution was allowed to stir at room temperature for 30 min, then cooled to −40 °C. Aqueous *tert*-butyl hydroperoxide solution of 14% (1 mL) was added dropwise, and the mixture stored at 4 °C overnight. The reaction was quenched with $NaHCO_3$ at 0 °C, poured into water, extracted 3× with EtOAc, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (5%→10% ethyl acetate/CH_2Cl_2) to yield 200 mg pure phosphoryl methyl ester **12** as a clear, colorless oil (93%). TLC 20% ethyl acetate/CH_2Cl_2 R_f=0.7 visualized with PMA. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08–7.17 (m, 4H), 3.61 (s, 3H), 3.52 (s, 2H), 1.44 (s, 18H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.7, 150.5, 130.1, 129.7, 119.9, 83.5, 51.8, 40.2, 29.7. ^{31}P NMR ($CDCl_3$) δ −40.8. ESI (+) LRMS (m/z): 716 $[M+Na]^+$.

*4.2.9. 2-(4-((Di-tert-butoxyphosphoryl)oxy) phenyl)acetic acid (**13**).* Methyl ester **12** (380 mg, 1.06 mmol) was dissolved in 5 mL H_2O and 13 mL THF, and cooled to 0 °C. A 0.3 M aqueous solution of LiOH (10.6 mL, 3.18 mmol) was added dropwise, and the mixture allowed to stir at 0 °C for 30 min. The reaction mixture was acidified with 1 M aqueous HCl to a pH of 3.0, after which it was extracted 3× with ethyl acetate, dried over anhydrous sodium sulfate, and concentrated to yield 365 mg carboxylic acid **13** as a white solid (quantitative). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.13–7.26 (m, 4H), 5.68 (br, 1H), 3.58 (s, 2H), 1.50 (s, 18H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.5, 150.4, 130.3, 129.6, 119.9, 84.0, 40.2, 29.8. ^{31}P NMR ($CDCl_3$) δ −41.1. ESI (+) HRMS (m/z): $[M+Na]^+$ calcd for $C_{16}H_{25}O_6P$, 367.1286; found, 367.1277.

*4.2.10. 4-(2-(((2R,5R,6R,Z)-6-Benzyl-2,5,7-tris((tert-butyldimethylsilyl)oxy)hept-3-en-1-yl)amino)-2-oxoethyl)phenyl di-tert-butyl phosphate (**14**).* Protected aminotriol **11** (19 mg, 0.027 mmol) was dissolved in 500 μL anhydrous CH_2Cl_2 and cooled to −78 °C. TBS-trifluoromethanesulfonate (8 μL, 0.030 mmol) was added dropwise, and the reaction mixture stirred at 0 °C for 2 h. Diisopropylethylamine (0.030 mmol, 5 μL) was then added dropwise, and the solution poured into H_2O and extracted 3× with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, concentrated in vacuo, and used immediately in the next step.

Phosphoryl acid **13** (11 mg, 0.032 mmol) was dissolved in 200 μL CH_2Cl_2 and stirred at 0 °C. EDC (6 mg, 0.032 mmol) was added, followed by HOBt hydrate (5 mg, 0.032 mmol) and DMAP (4 mg, 0.032 mmol). The resulting mixture was stirred for 20 min. The crude amine was dissolved in 200 μL CH_2Cl_2 and added to the acid solution dropwise. The reaction mixture was warmed to room temperature and allowed to stir for two days. The solvent was then removed in vacuo and the crude residue redissolved in ethyl acetate and washed with the following: water, 5% aqueous citric acid solution, satd aqueous $NaHCO_3$, and brine. The organic layer was then dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography (20%→30% ethyl acetate/hexanes) to yield 12 mg **14** as a light yellow oil (50% over two steps). TLC 20% ethyl acetate/hexane R_f=0.2 visualized with PMA. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.09–7.26 (m, 9H), 5.68 (br, 1H), 5.45–5.52 (m, 1H), 5.09–5.15 (m, 1H), 4.77 (d, J=9 Hz, 1H), 4.46 (br, 1H), 3.34–3.58 (m, 5H), 3.02–3.11 (m, 1H), 2.74–2.81 (m, 1H), 2.37–2.45 (m, 1H), 1.88 (br, 1H), 1.50 (s, 27H), 0.90 (s, 9H), 0.83 (s, 9H), 0.77 (s, 9H), −0.08–0.10 (m, 18H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3, 150.7, 141.1, 134.8, 130.5, 129.2, 128.9, 128.1, 125.6, 125.2, 120.3, 83.7, 67.9, 66.6, 61.6, 50.5, 45.3, 43.1, 31.1, 30.3, 29.8, 25.9, 25.6, 18.1, 17.7, −3.7, −4.0, −4.7, −5.0, −5.6. ^{31}P NMR ($CDCl_3$) δ −41.2. ESI (+) HRMS (m/z): $[M+Na]^+$ calcd for $C_{48}H_{86}NO_8PSi_3$, 942.5277; found, 942.5297.

*4.2.11. 4-(2-(((2R,5R,6R,Z)-6-Benzyl-2,5,7-trihydroxyhept-3-en-1-yl) amino)-2-oxoethyl)phenyl dihydrogen phosphate (**1**).* Acylation product **14** (23 mg, 0.025 mmol) was dissolved in 1 mL of a 1:1 mixture of anhydrous methanol and THF. The solution was cooled to 0 °C, and 230 μL of 3 M HCl in MeOH was added. The reaction mixture was warmed to room temperature and allowed to stir overnight. Solvents were then removed in vacuo, and the product triturated with hexane and ether, and co-evaporated 2× with toluene to yield 12 mg *cis*-enediol phosphate **1** (quantitative). $[\alpha]_D^{23}$ −20.6 (*c* 1.00, CH_3OH). ^{1}H NMR (CD_3OD, 300 MHz) δ 7.14–7.30 (m, 9H), 5.48–5.68 (m, 2H), 4.96 (br, phosphate), 4.52–4.56 (m, 2H), 3.20–3.66 (m, 10H), 2.59–3.21 (m, 2H), 1.79 (br, 1H). ^{13}C NMR (CD_3OD, 75 MHz) δ 150.3, 140.7, 133.9, 131.8, 131.3, 129.9, 128.8, 127.9, 125.4, 119.9, 67.4, 65.7, 59.5, 48.7, 45.0, 41.4, 31.8. ^{31}P NMR ($CDCl_3$) δ −30.1. ESI (+) HRMS (m/z): $[M+H]^+$ calcd for $C_{22}H_{28}NO_8P$ 466.1631; found, 466.1609.

Acknowledgements

Financial support from the National Cancer Institute (Grant R01 CA34619) and from the Purdue Research Foundation is gratefully

acknowledged. Support from the Purdue Cancer Center Support Grant P30 CA231168 for services provided by the NMR and Mass Spectrometry Shared Resources is appreciated.

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