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Phase Separation Macrocyclization in a Complex Pharmaceutical Setting: Application toward the Synthesis of Vaniprevir

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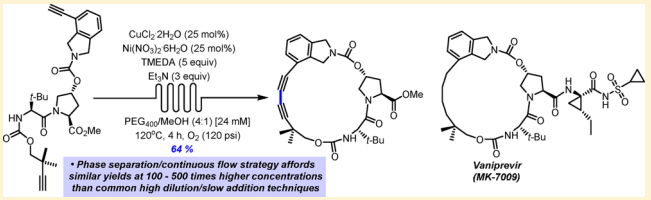
Supporting Information

ABSTRACT: A phase separation/continuous flow strategy employing an oxidative Glaser−Hay coupling of alkynes has been applied toward the synthesis of the macrocyclic core of complex pharmaceutical vaniprevir. The phase separation/continuous flow strategy afforded similar yields at 100−500 times the concentration and at shorter reaction times than common slow addition/high dilution techniques. In addition, dendritic PEG cosolvents were employed in the phase separation strategy for the first time and shown to allow productive macrocyclization at concentrations up to 200 mM.



INTRODUCTION

The macrocycle chemotype has made significant contributions to drug discovery due to unique properties that can allow for scanning of new chemical space.[1] Consequently, the interest in macrocycles in drug discovery has experienced significant growth in the past decade.[2] Terrett and co-workers have highlighted that current macrocyclic drugs are almost exclusively derived from naturally occurring macrocycles, with modifications typically occurring only at specific positions.[3] Synthetic macrocycles have come to represent a growing class of drug candidates, yet their diversity remains limited by the number of robust synthetic techniques amenable to library generation.[4] One of the major obstacles to any macrocycle synthesis involves surmounting the difficult ring closing event. Indeed, Terrett and co-workers also stated,"The only residual difficulty in macrocycle synthesis is finding conditions that allow good yields of cyclized materials from acyclic precursors.... For single compounds, this is usually achieved by resorting to the use of large reaction volumes and low reactant concentrations; conditions that were devised many years ago."[3] A representative example of the difficulty of optimizing a macrocyclization protocol was shown in the synthesis of the macrocyclic core of vaniprevir, a macrocyclic hepatitis C virus (HCV) NS3/4A protease inhibitor developed by Merck & Co. which was recently approved for treating hepatitis C in 2014 in Japan (Figure 1).[5] A macrolactamization protocol provided encouraging yields, but suffered from the use of stoichiometric reagents, while many catalytic cross-coupling routes provided undesirable yields. An olefin metathesis route proved optimal and could be conducted with low catalyst loading (0.2 mol %) at a concentration of 120 mM, although slow addition techniques were employed. Our group has reported a phase separation strategy as a novel macrocyclization strategy permitting catalytic transformations at relatively high concentrations.[6] The ability to control dilution effects rests upon the ability of poly(ethylene)glycol (PEG) cosolvents to form lipophilic aggregates in solution with an accompanying hydrophilic solvent, whereby the aggregates preferentially solubilize organic substrates. Slow diffusion of a linear precursor out of a PEG aggregate into the MeOH cosolvent and subsequent cyclization is believed to mimic slow addition conditions. Through judicious control of the ratio of PEG:MeOH, the diffusion of the linear precursor can be controlled to optimize the preference for macrocyclization versus oligomerization. In general, higher ratios of PEG:MeOH (2:1 → 8:1) allow for macrocyclization processes to be conducted at much higher concentrations all the while affording higher yields.

Consequently, the phase separation strategy represents an effort to apply the principles of sustainability to macrocyclization chemistry.[7] The phase separation strategy has recently been exploited by Itami and co-workers for the synthesis of thiophene-based macrocycles for materials science applications,[8] however no demonstrations of the utility of the phase separation strategy toward medicinal chemistry have been reported. Herein we report on the application of a phase separation strategy employing the rarely exploited Glaser−Hay coupling for the synthesis of a complex pharmaceutical target.

RESULTS AND DISCUSSION

Exploration of Different PEG Cosolvents for Macrocyclization via Phase Separation. The phase separation strategy relies upon the ability of PEG cosolvents to form lipophilic aggregates in MeOH solutions. Aggregation can be confirmed via surface tension measurements.[9] It has been shown that the structural characteristics of the PEG cosolvent can affect both its aggregation behavior and catalysis.[10] For

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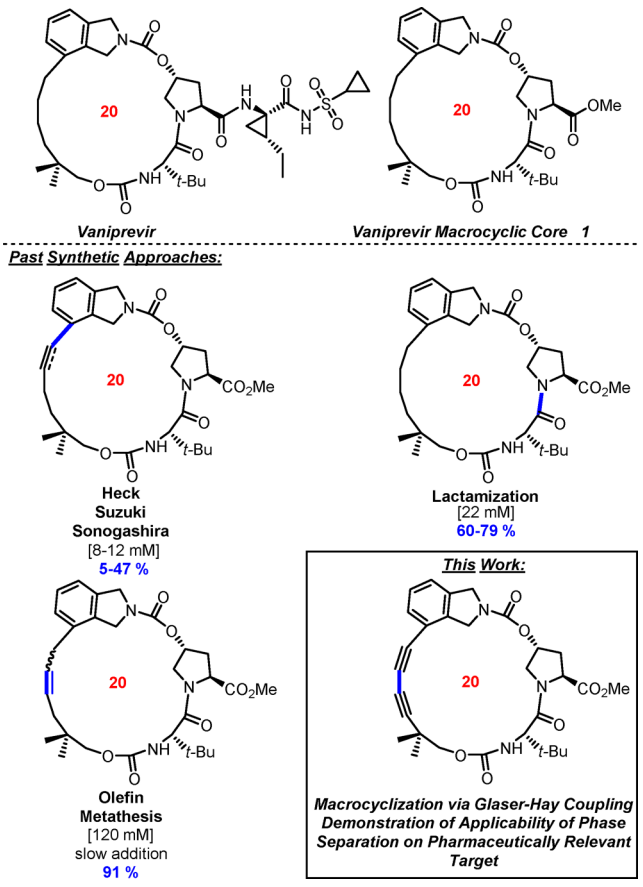

Figure 1. Macrocyclization strategies toward vaniprevir (ring size indicated in red).

example, hydroxyl-terminated PEGs are important for inducing aggregation in MeOH and the ability to observe high macrocyclization efficiency. More lipophilic solvents, such as longer chain PEGs, or poly(propylene)glycol (PPG) solvents, allow for catalysis at lower catalyst loadings.

The macrocyclization investigations via Glaser−Hay coupling were to conducted using reaction conditions previously developed by our group,[6] which exploit a Ni-based cocatalyst to accelerate the rate of oxidative coupling,[11] under microwave heating. Based upon previous reports,[6] PEG_{400} and PEG_{1450} were selected to be evaluated in the macrocyclization to form the core of vaniprevir (Figure 2). In addition, branched dendritic or "StarPEG" polymers were also explored for the first time in macrocyclizations via phase separation (Figure 2). Dendritic PEGs exhibit low cytotoxicities and have found use in drug delivery applications.[12] In addition, StarPEGs are known to have an enhanced ability to encapsulate hydrophilic and hydrophobic guests as well as sustained release in aqueous

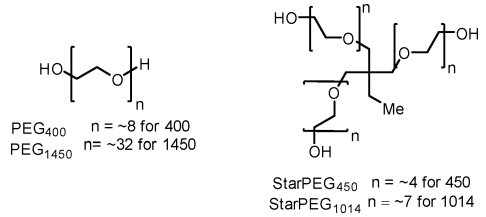

Figure 2. PEG cosolvents for study in a "phase separation" macrocyclization process.

media,[13] properties which would also be highly beneficial for developing a phase separation macrocyclization process. Two StarPEGs were chosen for study with average molecular weights of 450 and 1014 g·mol^{-1}. The StarPEGs were selected so that their behavior could be compared to linear PEGs that had similar average molecular weights (400 and 1450 respectively). Surface tension measurements demonstrated that StarPEGs exhibit aggregation behavior in MeOH similar to the other PEG cosolvents: aggregation occurred in ratios of 1:1 up to 8:1 StarPEG:MeOH.[14] Before exploring the macrocyclization of the vaniprevir precursor, preliminary studies were performed in batch to compare the ability of the selected PEG polymers to control dilution effects at concentrations of 24, 100, and 200 mM (Figure 3).[15] The macrocyclization of model bis-alkyne **2** was chosen for initial investigations as its aliphatic structure is relatively free of structural biases which could influence its ability to undergo macrocyclization. Control reactions in either pure MeOH or pure PEG solvent are important for establishing whether phase separation is proving beneficial to the macrocyclization process. Control macrocyclizations (**2** → **3**, Figure 3, top) performed in pure MeOH showed low yields of macrocycle **3** at 24 mM (22%), and only traces could be observed at either 100 or 200 mM. Control reactions performed in 100% PEG_{400}, $StarPEG_{450}$, or PEG_{1450}, at 24 mM, all afforded yields of macrocycle **3** below 5%. In sum, the control reactions all establish that phase separation provided by the mixtures of PEG cosolvent:MeOH is beneficial. An exception was the macrocyclization in $StarPEG_{1014}$, where the yield of **3** was 43%, implicating that, for phase separation to be successful in the dendritic PEG system, yields of **3** would have to exceed 43%. Also of note: macrocyclization in 100% PEG at 100 or 200 mM was not possible due to limited solubility of the catalysts and/or bis-alkyne **2**. The first macrocyclizations in PEG polymer:MeOH mixtures were performed at 24 mM at a ratio of 2:1 PEG polymer:MeOH, which had previously been shown to provide high yields of products (Figure 3, bottom). When macrocyclization was carried out in previously reported 2:1 PEG_{400}:MeOH mixture, the yield of **3** was 77%, and a similar yield of 78% was obtained for the analogous 2:1 $StarPEG_{450}$/MeOH. When using the linear 2:1 PEG_{1450}:MeOH mixture, the yield of **3** was 68%, but increased dramatically in 2:1 $StarPEG_{1014}$:MeOH to afford a 91% yield of macrocycle **3**.

Next, macrocyclization was performed at concentrations of 100 and 200 mM with 8 h reaction times, as the preliminary results demonstrated that macrocyclizations in the StarPEG solvents often proceeded more slowly. When macrocyclization was carried out in previously reported 2:1 PEG_{400}:MeOH mixture, yields of **3** dropped from 77% (24 mM) to 22% when the concentration was pushed to 200 mM. Reactions performed in the analogous 2:1 $StarPEG_{450}$/MeOH mixture provided slightly higher yields, however at the target 200 mM concentration, only 32% of **3** could be isolated. Further improvements were observed when PEG polymers with higher average molecular weights were used as cosolvents. In the linear 2:1 PEG_{1450}:MeOH mixture, the initial yield of **3** at 24 mM was only 68%, however the yield dropped much less appreciably when the macrocyclization was performed at 200 mM (40% of **3**). Finally, the best PEG polymer mixture surveyed was the 2:1 $StarPEG_{1014}$:MeOH, where at 24 mM a 91% yield of macrocycle **3** was isolated and at 200 mM a 64% yield was obtained.

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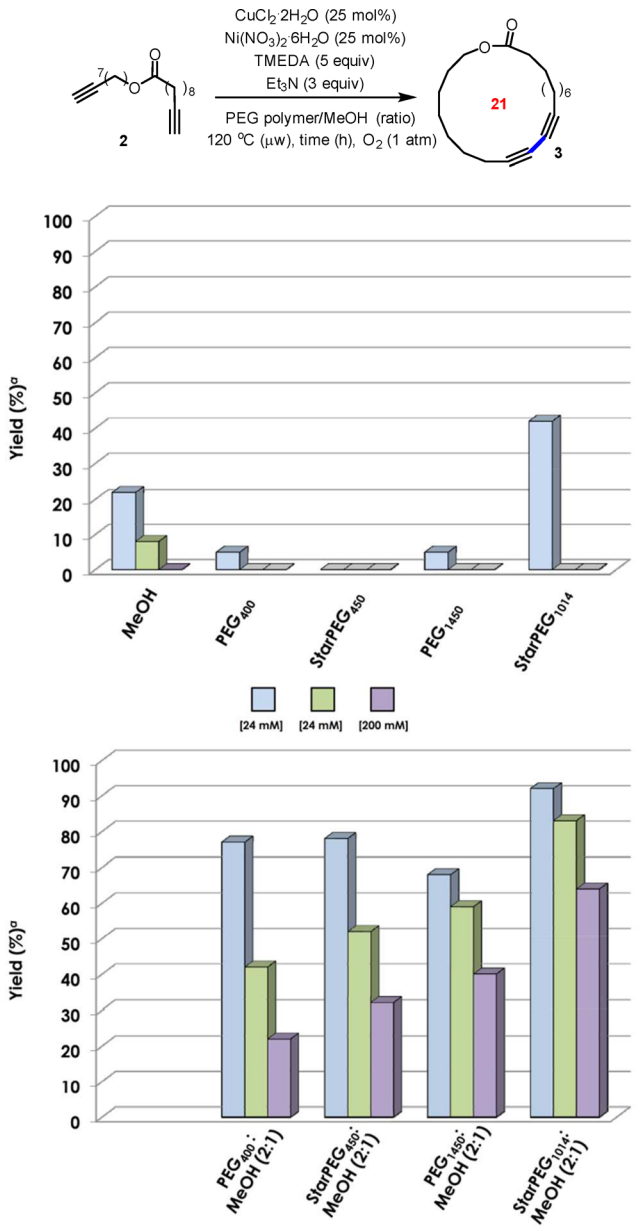
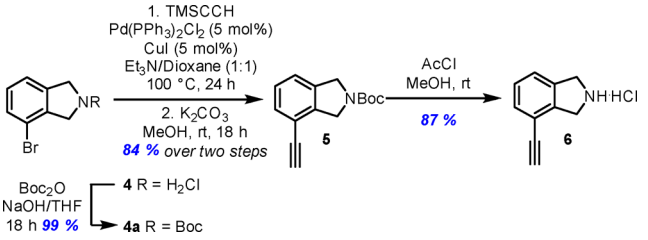


Figure 3. Effect of solvent and concentration on macrocyclization (**2** → **3**). Top: In pure MeOH, linear PEG, and StarPEG solvents. Bottom: Solvent mixtures of linear PEGs and StarPEGs in MeOH. Macrocyclizations were performed at 24 mM (blue, 6 h), 100 mM (green, 8 h), and 200 mM (purple, 8 h). [a]All entries represent isolated yields following silica gel chromatography. Ring size indicated in red.

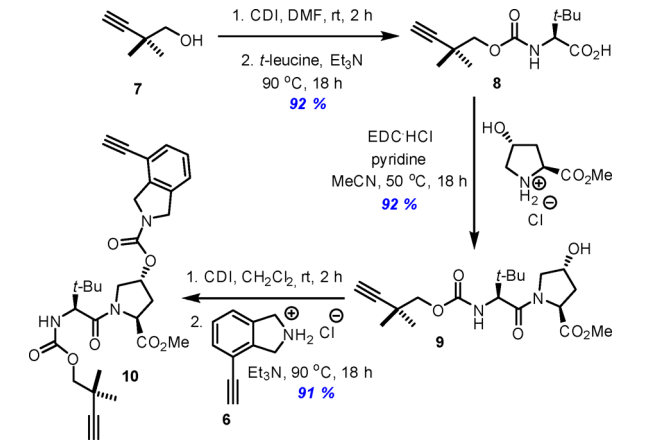
Synthesis of the Bis-Alkynyl Macrocyclization Precursor. The exploration of using a phase separation strategy employing a Glaser–Hay coupling began with the synthesis of the required alkynyl building block isoindoline **6** (Scheme 1).

The isoindoline **6** could be prepared from the commercially available bromide **4** via Boc protection, Sonogashira coupling, and deprotection of the trimethylsilyl and Boc protecting groups. The remaining peptidic portion of the macrocyclic precursor **10** was prepared from the known alkynyl alcohol **7**,[16] which was converted to the carbamate **8** in 92% yield using CDI and *tert*-leucine (Scheme 2). The following amide linkage was formed via coupling of the methyl ester of hydroxyproline to afford the dipeptide **9** in 92% yield. Lastly, a CDI-mediated

Scheme 1. Synthesis of Isoindoline 6



Scheme 2. Synthesis of an Acyclic Diyne Precursor 10



coupling with the isoindoline **6** afforded the desired macrocyclization precursor **10** in 91% yield.

Exploration of Different PEG Cosolvents for Macrocyclization toward the Core of Vaniprevir. Following the survey of different PEG:MeOH solvent mixtures for the macrocyclization of the model bis-alkyne **2**, a similar survey was undertaken for the cyclization of bis-alkyne **10** toward the macrocyclic core of vaniprevir **11**. With the goal of maximizing the efficiency of the process, it was decided to explore continuous flow methods for macrocyclization (**10** → **11**).[17]

It had already been demonstrated that the phase separation strategy for macrocyclization was amenable to continuous flow and that the associated efficient energy and mass transfer resulted in improved reaction times and yields.[18] As such, the macrocyclization of bis-alkyne **10** was performed by injecting the entire reaction mixture into a flow system consisting of two stainless steel reactors in series heated to 120 °C. As the Glaser–Hay reaction is an oxidative process, the first of the reactors was a tube-in-tube reactor equipped to saturate the reaction mixture with oxygen.[19] It is important to note that the bis-alkyne **10** is structurally much more complex than the model bis-alkyne **2**. It is difficult to predict *a priori* whether the additional functionalization in **10** creates a bias toward productive macrocyclization, or undesired dimerization or oligomerization.[20] The first macrocyclizations (**10** → **11**) investigated were control reactions performed in 100% MeOH at three different concentrations (Figure 4, top). The macrocyclization at 24 mM afforded low yields (29% of **11**), and slightly lower yields were obtained at 50 mM (22%) and 100 mM (24%).[21] Next, a second set of control reactions were performed whereby macrocyclization was conducted in 100% PEG at 24 mM. For macrocyclization in PEG_{400}, the isolated yield of macrocycle **11** was low (13%), but for the analogous dendritic $StarPEG_{450}$, the yield was again much greater (43% of **11**), demonstrating the increased reactivity observed with more

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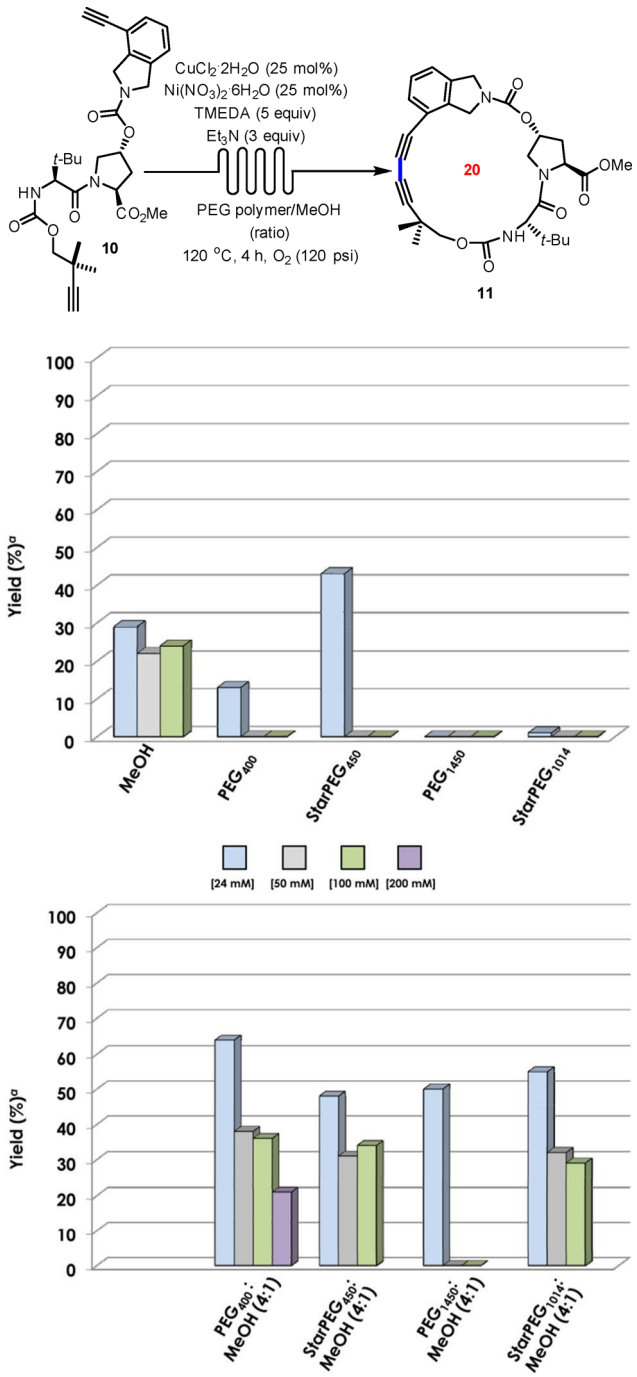


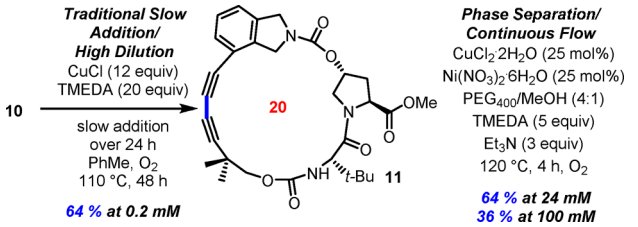
Figure 4. Effect of solvent and concentration on macrocyclization (**10** → **11**). Top: In pure MeOH, linear PEG, and StarPEG solvents. Bottom: Solvent mixtures of linear PEGs and StarPEGs in MeOH. Macrocyclizations were performed at 24 mM (blue), 50 mM (gray), 100 mM (green), and 200 mM (purple). [a]All entries represent isolated yields following silica gel chromatography. Ring size indicated in red.

lipophilic PEG solvents. In contrast, macrocyclizations were very inefficient when higher molecular weight PEGs were used. The linear PEG_{1450} is a solid at room temperature, making the reaction under flow conditions difficult to perform, while, for the dendritic $StarPEG_{1014}$, a liquid at room temperature, only trace amounts of the macrocycle **11** could be isolated. Unfortunately, macrocyclizations at 100 mM could not be performed due to problematic solubility of substrates and/or catalysts in the neat PEG solvents.

Next, the macrocyclization was investigated in the PEG:MeOH mixtures (Figure 4, bottom). Given the higher yields observed with $StarPEG_{1014}$ obtained in the macrocyclization of the simple model macrocycle **11**, the first cyclization toward the vaniprevir core **11** was investigated in 2:1 $StarPEG_{1014}$:MeOH (24 mM).[14] When **10** was subjected to macrocyclization in 2:1 $StarPEG_{1014}$:MeOH, only a 31% yield of macrocycle **11** was obtained, with complete conversion of the starting bis-alkyne **10**. Reasoning that a better preference for macrocyclization could be obtained at higher PEG:MeOH ratios, the macrocyclizations of bis-alkyne **10** were conducted at 4:1 $StarPEG_{1014}$:MeOH, which afforded a yield of 55% of **11**. Increasing or decreasing the ratio of PEG:MeOH did not improve the conversion to a significant degree,[22] and a ratio of 4:1 PEG polymer:MeOH was used for all further investigations. When comparing $StarPEG_{1014}$ to the linear analogue PEG_{1450}, it was found that the yield of macrocycle **11** was slightly lower (50%) at 4:1 PEG_{1450}:MeOH. A lower molecular weight $StarPEG_{450}$ also gave a comparable yield of **11** (48% at 4:1 $StarPEG_{450}$:MeOH). However, the best yield of **11** at 24 mM was with a 4:1 mixture of PEG_{400}:MeOH (64%).[23] Next, attempts were made to increase the concentration of the reaction to both 50 and 100 mM. After surveying 4:1 mixtures of PEG_{400}, $StarPEG_{450}$, and $StarPEG_{1014}$ with MeOH, the best yield at either concentration was obtained with 4:1 PEG_{400}:MeOH (38% of macrocycle **11** at 50 mM and 36% of macrocycle **11** at 100 mM).[24]

In an effort to compare the phase separation/continuous flow protocol to common slow addition/high dilution strategies, the bis-alkyne **10** was cyclized at low concentration (0.2 mM) (Scheme 3). Excess copper/ligand was used to promote

Scheme 3. Comparing Slow Addition/High Dilution and Phase Separation/Continuous Flow Strategies toward the Vaniprevir Core 11[a]



[a]Ring size indicated in red.

macrocyclization in 64% yield of **11** with a reasonable reaction time (48 h).[25] Consequently, the phase separation/continuous flow strategy provided similar yields at concentrations greater than 100 times that of slow addition/high dilution strategies. In addition, the former could promote macrocyclization at 36% yield at up to 500 times greater concentrations. The macrocyclic diyne **11** could be hydrogenated to afford the same macrocyclic intermediate **1** obtained by the Merck research team (Scheme 4).

In summary, the phase separation strategy has been applied for the first time to macrocyclization of a complex pharmaceutical target, the macrocyclic core of vaniprevir. The phase separation strategy demonstrated good functional group tolerance to the nitrogen-based heterocycles, dipeptides, and carbamates embedded within the structure of vaniprevir and provided good yields of the desired macrocyclic core **11** (55–64%) using either PEG_{400}:MeOH or newly explored

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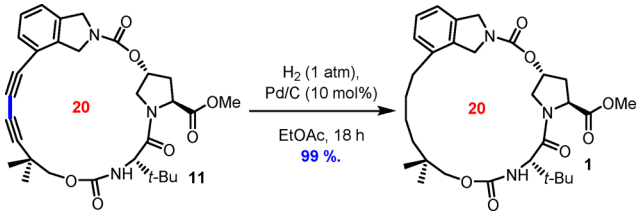
Scheme 4. Completing the Synthesis of the Vaniprevir Core 1[a]



[a]Ring size indicated in red.

StarPEG$_{1014}$:MeOH. In addition, the macrocyclization demonstrates the utility of the Glaser–Hay oxidative coupling of alkynes for macrocyclization of pharmaceuticals. Macrocyclization toward the core of vaniprevir could be conducted at 100 mM using linear or dendritic PEG cosolvents. A "simpler" macrocyclization on an unbiased substrate could be conducted at 200 mM in good yields (64% of **3**) using the newly explored dendritic PEGs as cosolvents. As a number of other synthetic processes rely upon controlling concentration effects,[26] it is possible that the new dendritic PEG/solvent mixtures could be used to improve such processes. It is also expected that as the interest in macrocycles in drug discovery continues to grow, so will the need for macrocyclization techniques that strive toward sustainability.

EXPERIMENTAL SECTION

All reactions that were carried out under anhydrous conditions were performed under an inert argon or nitrogen atmosphere in glassware that had previously been dried overnight at 120 °C or had been flame-dried and cooled under a stream of argon or nitrogen.[27] All chemical products were obtained from Sigma-Aldrich Chemical Co. or Alfa Aesar and were reagent quality. Technical solvents were obtained from VWR International Co. or ACP Chemicals Inc. Anhydrous solvents (CH_2Cl_2, Et_2O, THF, DMF, toluene, and *n*-hexane) were dried and deoxygenated using a GlassContour system (Irvine, CA). Bis-alkyne **2**,[6a] alcohol **7**,[28] and hydroxyproline methyl ester[29] were synthesized according to the literature. Isolated yields reflect the mass obtained following flash column silica gel chromatography. Organic compounds were purified using the method reported by W. C. Still[30] and using silica gel obtained from Silicycle Chemical division (40–63 nm; 230–240 mesh). Analytical thin-layer chromatography (TLC) was performed on glass-backed silica gel 60 coated with a fluorescence indicator (Silicycle Chemical division, 0.25 mm, F_{254}.). Visualization of TLC plate was performed by UV (254 nm), $KMnO_4$, or *p*-anisaldehyde stains. All mixed solvent eluents are reported as v/v solutions. Concentration refers to removal of volatiles at low pressure on a rotary evaporator. All reported compounds were homogeneous by thin layer chromatography (TLC) and by ^{1}H NMR. NMR spectra were taken in deuterated $CDCl_3$ using Bruker AV-300 and AV-400 instruments unless otherwise noted. Signals due to the solvent served as the internal standard ($CHCl_3$: δ 7.27 for ^{1}H, δ 77.0 for ^{13}C). The ^{1}H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); the list of coupling constants (J) corresponds to the order of the multiplicity assignment. Mass spectrometric analyses for nominal masses were performed on a quadrupole analyzer, while high resolution masses were performed on a TOF analyzer.

***tert*-Butyl 4-Bromoisoindine-2-carboxylate (4a).** To a solution of 4-bromoisoindoline hydrochloride (**4**) (2.64 g, 11.2 mmol, 1 equiv) in NaOH 1 M (26 mL) and THF (26 mL) was added Boc_2O (2.71 g, 12.4 mmol, 1.1 equiv). The mixture was stirred for 16 h at room temperature. EtOAc and H_2O were added to the mixture, and the layers were separated. The aqueous phase was extracted 2× with EtOAc. The organic phases were combined, washed with brine, dried with Na_2SO_4, and concentrated in vacuo. The crude solid (3.3 g, 99%) was clean. *Note that rotamers are formed and can result in complex splitting patterns in the ^{1}H NMR, or can cause doubling of some peaks in the ^{13}C NMR spectrum. For clarity, all peaks are reported.* ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.39 (d, J = 8.6 Hz, 1 H), 7.24–7.08 (m, 2 H), 4.80–4.57 (m, 4 H), 1.58–1.45 (m, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$,) δ ppm = 154.3, 139.0, 138.8, 138.0, 137.9, 130.29, 130.25, 129.2, 129.1, 121.5, 121.3, 117.6, 117.3, 85.1, 80.0, 79.9, 53.5, 53.4, 53.2, 52.9, 28.5, 27.4; HRMS (ESI) m/z calculated for $C_{13}H_{16}BrNO_2Na$ $[M + Na]^+$, 320.0257; found, 320.0265.

***tert*-Butyl 4-((Trimethylsilyl)ethynyl)isoindoline-2-carboxylate (S1).** In a sealed tube, **4a** (167 mg, 0.56 mmol, 1 equiv) was dissolved in Et_3N (1.5 mL) and 1,4-dioxane (1.5 mL). The solution was degassed with N_2 for 5 min. $Pd(PPh_3)_2Cl_2$ (39.3 mg, 0.056 mmol, 0.1 equiv) and CuI (5.3 mg, 0.028 mmol, 0.05 equiv) were added, and the solution was degassed a second time with N_2 for 5 min. Ethynyltrimethylsilane (0.39 mL, 2.8 mmol, 5 equiv) was added, and the mixture was stirred for 24 h at 100 °C. Upon completion, the mixture was passed through a pad of Celite and concentrated *in vacuo*. Purification by silica gel chromatography (3% EtOAc/hexanes) gave the desired product as an off-white solid. The product may be contaminated with residual butadiyne byproduct from homocoupling of the ethynyltrimethylsilane. As such, yields were calculated following the purification of product **5** below. *Note that rotamers are formed and can result in complex splitting patterns in the ^{1}H NMR, or can cause doubling of some peaks in the ^{13}C NMR spectrum. For clarity, all peaks are reported.* ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37–7.32 (m, 1 H), 7.25–7.15 (m, 2 H), 4.78–4.66 (m, 4 H), 1.54–1.50 (m, 9 H), 0.29–0.23 (m, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm = 154.4, 140.3, 139.9, 137.2, 136.9, 130.2, 130.1, 127.4, 127.3, 122.8, 122.5, 118.3, 188.0, 102.0, 101.6, 98.8, 79.7, 79.7, 52.7, 52.4, 52.3, 52.1, 28.5, 28.4, −0.1; HRMS (ESI) m/z calculated for $C_{18}H_{25}NO_2SiNa$ $[M + Na]^+$, 338.1548; found, 338.1547.

***tert*-Butyl 4-Ethynylisoindoline-2-carboxylate (5).** To a solution of indoline **S1** (1.711 g, 5.43 mmol, 1 equiv) in MeOH (40 mL) was added K_2CO_3 (3.75 g, 27.15 mmol, 5 equiv). The solution was stirred for 18 h at room temperature. Upon completion by TLC, EtOAc and H_2O were added and the layers were separated. The aqueous phase was extracted with EtOAc (2×). The organic phases were combined, washed with brine, dried with Na_2SO_4, and concentrated *in vacuo*. Purification by silica gel chromatography (2.5% EtOAc/hexanes) gave the terminal alkyne (1.010 g, 84%) as a white solid. *Note that rotamers are formed and can result in complex splitting patterns in the ^{1}H NMR, or can cause doubling of some peaks in the ^{13}C NMR spectrum. For clarity, all peaks are reported.* ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40–7.35 (m, 1 H), 7.26–7.18 (m, 2 H), 4.78–4.66 (m, 4 H), 3.30–3.24 (m, 1 H), 1.55–1.51 (m, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm = 154.4, 154.3, 140.4, 140.2, 137.5, 137.2, 130.8, 130.7, 127.5, 127.4, 123.2, 122.9, 117.2, 117.0, 81.3, 81.1, 80.7, 80.5, 79.8, 79.8, 52.7, 52.4, 52.2, 52.1, 28.5; HRMS (ESI) m/z calculated for $C_{15}H_{17}NO_2Na$ $[M + Na]^+$, 266.1152; found, 266.1152.

4-Ethynylisoindoline Hydrochloride (6). To a solution of isoindoline **5** (786 mg, 3.23 mmol, 1 equiv) in MeOH (15 mL) was added dropwise AcCl (0.86 mL, 16.17 mmol, 5 equiv) at 0 °C. The resulting mixture was stirred for 18 h (or until completed by TLC) at room temperature. Et_2O was added and a gray precipitate was formed and filtered, washed with Et_2O (2×), and dried under vacuum to give the desired isoindoline salt as a gray powder (507 mg, 87%). ^{1}H NMR (400 MHz, MeOD-d_4) δ = 7.52–7.38 (m, 3 H), 4.70–4.66 (m, 4 H), 3.93 (s, 1 H); ^{13}C NMR (100 MHz, MeOD-d_4) δ ppm = 138.3, 136.3, 133.3, 130.6, 124.7, 119.1, 84.5, 80.8, 52.7, 49.8; HRMS (ESI) m/z calculated for $C_{10}H_{10}N$ $[M + H]^+$, 144.0812; found, 144.0808.

(*S*)-2-((((2,2-Dimethylbut-3-yn-1-yl)oxy)carbonyl)amino)-3,3-dimethylbutanoic Acid (8). In a sealed tube, 2,2-dimethylbut-3-yn-1-ol (**7**) (1.84 g, 18.7 mmol, 1 equiv) and 1,1′-carbonyldiimidazole (3.96 g, 24.4 mmol, 1.3 equiv) were dissolved in DMF (20 mL). The mixture was stirred 2 h at room temperature. Then, L-*tert*-leucine (3.21 g, 24.4 mmol, 1.3 equiv) and Et_3N (3.74 mL, 26.3 mmol, 1.4 equiv) were added, and the resulting mixture was warmed to 90 °C and

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stirred for 16 h. Then reaction was then cooled back to room temperature, and MTBE and NaOH 0.5 M were added. The layers were separated, and the organic layer was discarded. MTBE was added to the aqueous phase, and the pH was adjusted to 1 using HCl 6 M. The layers were separated again. The organic phase was washed with brine, dried with $MgSO_4$, and concentrated *in vacuo*. The crude carboxylic acid (4.4 g, 92%) was obtained as a sticky semisolid. $[\alpha]_D^{25}$ = 4.0 (c = 0.0030, MeOH). *Note that rotamers are formed and can result in complex splitting patterns in the ^1H NMR, or can cause doubling of some peaks in the ^{13}C NMR spectrum. For clarity, all peaks are reported.* ^1H NMR (300 MHz, $CDCl_3$) δ = 11.22 (br s, 1 H), 6.50 (d, J = 7.8 Hz, 0.3 H), 5.43 (d, J = 9.5 Hz, 0.7 H), 4.19 (d, J = 9.5 Hz, 0.7 H), 4.05–3.90 (m, 2.3 H), 2.11 (s, 1 H), 1.22 (s, 6 H), 1.01 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm = 176.2, 157.0, 156.3, 88.7, 88.1, 72.8, 72.0, 69.1, 68.9, 63.2, 62.1, 34.6, 33.9, 31.7, 26.4, 25.6; HRMS (ESI) m/z calculated for $C_{13}H_{21}NO_4Na$ $[M + Na]^+$, 278.1365; found, 278.1363.

Methyl (2*S*,4*R*)-1-((*S*)-2-((((2,2-Dimethylbut-3-yn-1-yl)oxy)carbonyl)amino)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxylate (9). Carboxylic acid (**8**) (1.03 g, 3.48 mmol, 1 equiv) was dissolved in MeCN (3 mL) at room temperature. *trans*-4-Hydroxy-L-proline methyl ester hydrochloride (695 mg, 3.83 mmol, 1.1 equiv) and pyridine (0.42 mL, 5.22 mmol, 1.5 equiv) were added to the stirring solution. EDC-HCl (900 mg, 4.70 mmol, 1.35 equiv) was added last, and the mixture became bright yellow. The reaction was warmed to 50 °C and stirred for 16 h. The crude mixture was cooled back to room temperature, and PhMe and an aqueous solution of citric acid (15 wt %) were added. The mixture was stirred for 5 min, and the aqueous layer was discarded. Brine was added, and the resulting mixture was stirred for an additional 5 min. The phases were separated, and the organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The crude solid was azeotroped with PhMe, and the clean alcohol was obtained as a colorless oil (1.23 g, 92%). $[\alpha]_D^{25}$ = −67.3 (c = 0.0055, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ = 5.49 (d, J = 9.1 Hz, 1 H), 4.69 (t, J = 8.7 Hz, 1 H), 4.60–4.51 (m, 1 H), 4.27 (d, J = 8.9 Hz, 1 H), 4.06–3.97 (m, 2 H), 3.93–3.85 (m, 1 H), 3.79–3.73 (m, 1 H), 3.75 (s, 3 H), 2.41–2.31 (m, 1 H), 2.13 (s, 1 H), 2.09–1.98 (m, 1 H), 1.24 (s, 6 H), 1.06 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm = 172.4, 170.6, 156.4, 88.5, 71.8, 69.8, 69.0, 59.0, 57.7, 56.3, 52.0, 37.3, 35.8, 31.5, 26.0, 25.5; HRMS (ESI) m/z calculated for $C_{19}H_{31}N_2O_6$ $[M + H]^+$, 383.2184; found, 383.2182.

(3*R*,5*S*)-1-((*S*)-2-((((2,2-Dimethylbut-3-yn-1-yl)oxy)carbonyl)-amino)-3,3-dimethylbutanoyl)-5-(methoxycarbonyl)-pyrrolidin-3-yl 4-ethynylisoindoline-2-carboxylate (10). Alcohol **9** (946 mg, 2.48 mmol, 1 equiv) and 1,1′-carbonyldiimidazole (CDI) (522 mg, 3.22 mmol, 1.3 equiv) were dissolved in dry DCM (12 mL). The mixture was stirred for 2 h at room temperature. Then, isoindoline (**6**) (579 mg, 3.22 mmol, 1.3 equiv) and Et_3N (1.03 mL, 7.43 mmol, 3 equiv) were added, and the resulting mixture was warmed to 50 °C and stirred for 18 h. Then the reaction was diluted with DCM, and the phases were separated. The organic layer was washed with HCl 1 M (2×), $NaHCO_{3(satd)}$, and brine. The organic phase was dried with Na_2SO_4 and concentrated *in vacuo*. Purification by silica gel chromatography (40% EtOAc/hexanes gave the desired bis-alkyne (1.25 g, 91%) as a white solid. Mp: 72.2 °C; $[\alpha]_D^{25}$ = −14.8 (c = 0.00135, MeOH). *Note that rotamers are formed and can result in complex splitting patterns in the ^1H NMR, or can cause doubling of some peaks in the ^{13}C NMR spectrum. For clarity, all peaks are reported.* ^1H NMR (500 MHz, $CDCl_3$) δ = 7.41–7.35 (m, 1H), 7.26–7.17 (m, 2H), 5.43–5.37 (m, 2H), 4.83–4.61 (m, 4H); 4.24 (d, J = 9.5 Hz, 1H), 4.20 (t, J = 12.5 Hz, 1H), 3.94–3.84 (m, 1H), 3.80 (d, J = 10.3 Hz, 1H), 3.77–3.75 (m, 3H), 3.55–3.46 (m, 1H), 3.32–3.27 (m, 1H), 2.55–2.48 (m, 1H), 2.26–2.18 (m,1H), 2.06 (d, J = 7.5 Hz, 1H), 1.26–1.22 (m, 1H), 1.13–1.06 (m, 6H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm = 172.0, 171.05, 171.00, 156.3, 153.8, 153.7, 139.8, 139.6, 136.9, 136.5, 131.0, 130.9, 127.71, 127.67, 123.1, 122.9, 117.3, 117.2, 88.8, 88.7, 81.9, 81.5, 80.4, 73.5, 71.68, 71.65, 68.73, 68.68, 59.2, 59.1, 57.9, 57.8, 54.03, 53.95, 53.0, 52.5, 52.3, 52.0, 35.4, 35.3, 35.0, 34.8, 31.5, 31.4, 26.2, 25.6, 25.5; HRMS (ESI) m/z calculated for $C_{30}H_{38}N_3O_7$ $[M + H]^+$, 552.2710; found, 552.2704.

Macrocycle (11). Slow Addition Procedure. To a 1 L triple-neck round-bottom flask equipped with a stirring bar and a condenser, CuCl (119 mg, 1.2 mmol, 12 equiv) and TMEDA (0.3 mL, 2.0 mmol, 20 equiv) were added to PhMe (405 mL). The mixture was stirred and warmed at 110 °C. To the mixture, a solution of bis-alkyne **10** (55.2 mg, 0.1 mmol, 1 equiv) in PhMe (50 mL) was slowly added over 24 h (0.035 mL/min) with a syringe pump. The mixture was stirred and heated for an additional 24 h. The reaction was then cooled down to room temperature and concentrated under reduced pressure. Flash chromatography was performed (40 → 60% EtOAc in hexanes) to afford the desired product as a white solid (35 mg, 64%). Mp = 136.4 °C; $[\alpha]_D^{25}$ = −32.0 (c = 0.0020, MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ = 7.30–7.26 (m, 1H), 7.25–7.21 (m, 2H), 5.71 (d, J = 9.1 Hz, 1H), 5.30–5.26 (m, 1H), 4.83 (d, J = 10.5 Hz, 1H), 4.76–4.73 (m, 2H), 4.64–4.59 (m,2H), 4.59–4.56 (m, 1H), 4.43 (d, J = 9.1 Hz, 1H), 4.07 (dd, J = 11.8, 1.6 Hz, 1H), 3.85 (dd, J = 11.7, 3.6 Hz, 1H), 3.76 (s, 3H), 3.29 (d, J = 10.5 Hz, 1H), 2.75–2.69 (m, 1H), 2.16–2.09 (m, 1H), 1.34 (s, 3H), 1.23 (s, 3H), 1.06 (s, 9H); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ ppm = 172.0, 170.4, 155.3, 153.3, 144.4, 136.2, 129.4, 127.9, 123.1, 117.1, 89.6, 78.7, 74.3, 72.2, 71.4, 64.7, 59.4, 57.7, 53.8, 52.8, 52.3, 52.1, 37.2, 35.6, 33.1, 26.3, 25.4, 25.3; HRMS (ESI) m/z calculated for $C_{30}H_{36}N_3O_7$ $[M + H]^+$, 550.2565; found, 550.2549.

Microwave Procedure. In an open microwave vial equipped with a stirring bar, bis-alkyne **10** (26.5 mg, 0.048 mmol, 1 equiv), $CuCl_2 \cdot 2H_2O$ (2.0 mg, 0.012 mmol, 25 mol %), and $Ni(NO_3)_2 \cdot 6H_2O$ (3.5 mg, 0.012 mmol, 25 mol %) were dissolved in MeOH (0.4 mL), and the mixture was stirred at room temperature for 30 s or until the metals were solubilized. Poly(ethylene) glycol 1450 (1.6 mL), TMEDA (0.035 mL, 0.24 mmol, 5 equiv), and Et_3N (0.02 mL, 0.144 mmol, 3 equiv) were added, and the mixture was stirred at room temperature for an additional 30 s. The vial was then sealed with a microwave cap. The reaction was warmed to 120 °C for 6 h. The crude mixture was purified by chromatography (40 → 60% EtOAc in hexanes) to afford the desired product as a white solid (13.7 mg, 50%).

Continuous Flow Procedure. In a 4 mL reaction vial equipped with a stirring bar, bis-alkyne **10** (24.4 mg, 0.048 mmol, 1 equiv), $CuCl_2 \cdot 2H_2O$ (2.0 mg, 0.012 mmol, 25 mol %), and $Ni(NO_3)_2 \cdot 6H_2O$ (3.5 mg, 0.012 mmol, 25 mol %) were dissolved in MeOH (0.4 mL), and the mixture was stirred at room temperature for 30 s or until the metals were solubilized. PEG cosolvent (1.6 mL), TMEDA (0.035 mL, 0.24 mmol, 5 equiv), and Et_3N (0.02 mL, 0.144 mmol, 3 equiv) were added, and the mixture was stirred at room temperature for an additional 30 s and then taken up into a syringe. The reaction mixture was injected using a 2 mL injection loop into the flow reactor for a reaction time of 240 min (1 × 15 mL stainless steel reactor (tube-in-tube, O_2 (120 psi)) and 1 × 10 mL stainless steel reactor with a 32 cm length section of stainless steel tubing between reactors) at a flow rate of 0.104 mL/min at 120 °C. The flow reaction was conducted in a Vapourtec R4 reactor and an R2+ pumping module. The continuous flow setup is ended with a back pressure regulator (IDEX 250 psi). Upon completion, silica gel was added to the collection flask and the volatiles were removed under vacuum. The crude mixture was purified by chromatography (40 → 60% EtOAc in hexanes) to afford the desired product as a white solid (15.5 mg, 64%).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b01308.

Surface tension measurements, macrocyclization data in tabular form, and spectroscopic data for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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