Tetrahedron 69 (2013) 5744–5750

Contents lists available at SciVerse ScienceDirect

Tetrahedron

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

Cationic dirhodium carboxylate-catalyzed synthesis of dihydropyrimidones from propargyl ureas

Miao Yang [a], Shannon J. Odelberg [b,c,d], Zongzhong Tong [d], Dean Y. Li [b,c,e,f], Ryan E. Looper [a,*]

[a] Department of Chemistry, University of Utah, Salt Lake City, UT 84112, United States
[b] Department of Medicine, Cardiology Division, University of Utah, Salt Lake City, UT 84132, United States
[c] Program in Molecular Medicine, University of Utah, Salt Lake City, UT 84112, United States
[d] Navigen Inc., Salt Lake City, UT 84108, United States
[e] Department of Oncology, University of Utah, Salt Lake City, UT 84112, United States
[f] The Key Laboratory for Human Disease Study of Sichuan Province, Institute of Laboratory Medicine, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, Sichuan 610072, China

ARTICLE INFO

Article history:
Received 11 March 2013
Received in revised form 9 April 2013
Accepted 11 April 2013
Available online 19 April 2013

Keywords:
Dihydropyrimidone
Heterocycle
Hydroamination
Rhodium catalysis
ARF 6

ABSTRACT

Cationic Rh(II) complexes are able to catalyze the regioselective hydroamination of propargyl ureas in a 6-*endo* fashion. This transformation permits access to interesting substitution patterns of dihydropyrimidines, which have found use as nucleotide exchange factor inhibitors.

1. Introduction

Accessible through multicomponent reactions, the dihydropyrimidone scaffold has been prevalent in both pharmaceutical and academic screening campaigns for decades.[1] Given the cornucopia of biological activities reported for this heterocyclic core,[2] methodologies have developed advancing the vetted Biginelli condensation[3] to include enantioselective variants and orthogonal substitution patterns.[4,5] Recently Rovis and co-workers reported an enantioselective Rh(I) catalyzed [4+2] cycloaddition of α,β-unsaturated imines and isocyanates to generate dihydropyrimidones, highlighting the continuing need for methodologies to access this core.[6]

Our group has recently been interested in the cyclization of propargylguanidines, particularly from the vantage of controlling the cyclization in either a 5-*exo* or 6-*endo* fashion.[7] The application of this strategy to propargyl ureas introduces an added level of selectivity, that is, not only are 5-*exo* and 6-*endo* modes of cyclization operable but they can occur through either the urea nitrogen or oxygen (Fig. 1).

The cyclization of propargyl ureas and related propargylcarbamates has been extensively studied, most frequently using salts of gold,[8] silver,[9] palladium,[10] and copper.[11] Kinetically favored, cyclization to form the five-membered ring is most commonly observed, both with N- and O-connectivity. Recently Toste and coworkers described the isolation of the dihydrooxazinoneimine in significant quantities from the cationic Au(I)-catalyzed cyclization of *N*-Ts-propargyl ureas,[8o] but without fail the six-membered rings are the minor component of the cyclization event. To the best of our knowledge the cyclization of propargyl ureas, selectively in a 6-*endo* fashion through the urea nitrogen, has not been observed. Achieving this selectivity would greatly add to our ability to create new substitution patterns of a biomedically significant small molecule scaffold.

2. Results and discussion

2.1. Cationic Rh(II) cyclizations of propargyl ureas

Having noted the ability of dirhodium(II)-carboxylates to selectively catalyze the 6-*endo* cyclization of propargylguanidines, in

* Corresponding author. Tel.: +1 801 585 0408; e-mail address: r.looper@utah.edu (R.E. Looper).

0040-4020/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2013.04.071

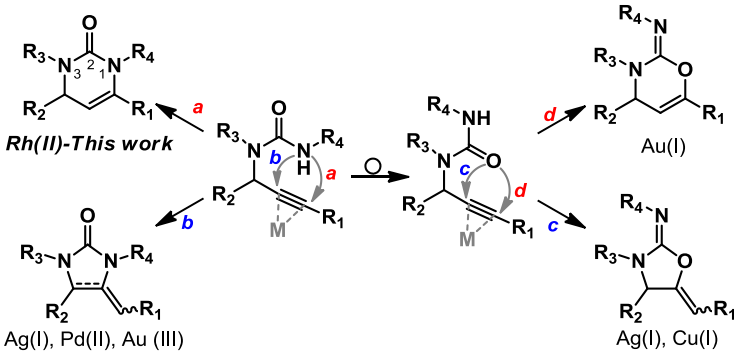
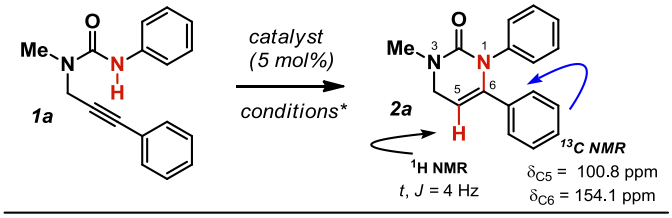


Fig. 1. Dihydropyrimidone strategy.

direct contrast to other π-Lewis acids, we were encouraged to explore the use of these catalysts in the cyclization of propargyl ureas.[7b] From our previous research AgOAc and $Rh_2(Oct)_4$ were preferred to selectively promote the 5-*exo* and 6-*endo* modes of cyclization, respectively.

We initially examined the ability of π-Lewis acids to catalyze the cyclization of propargylurea **1a** (Table 1). Surprisingly AgOAc failed to catalyze the cyclization at room temperature or 70 °C (entries 2 and 3) although Van der Eycken has recently reported that this transformation is possible with 20 mol % AgOTf at 110 °C, confirming that the propargyl ureas are much less nucleophilic than their di-Boc guanidine counterparts.[9e] More discouraging was the fact that $Rh_2(Oct)_4$ also failed to promote the cyclization (entries 4 and 5). The more Lewis-acidic $Rh_2(TFA)_4$ was then examined. While unreactive at room temperature (entry 6), formation of a single cyclization product (**2a**) occurred when the temperature was increased to 70 °C, albeit in 50% isolated yield (entry 7). Encouragingly, the product did appear to have cyclized in a 6-*endo* fashion as evidenced by the coupling constant of the vinylic proton at C5 (^3J=4.0 Hz). Further the ^{13}C chemical shifts of C5 and C6 were much more consistent with enamine connectivity versus that of an enol-ether. We anticipated that a suitable cationic Rh(II) complex, with more labile ligands, might lend the greater Lewis acidity and improve the reactivity. To test this assumption, we turned our attention to the cationic catalyst $[Rh_2(OAc)_2(MeCN)_6][BF_4]_2$.[12] To our

Table 1
Catalysts and solvents screen



Entry	Catalyst (5 mol %)	Solvent	Temp (°C)	Isolated yield (%) **2a**
1	N/A	CH_2Cl_2	80	NR
2	AgOAc	$HOAc/CH_2Cl_2$	rt	NR
3	AgOAc	$HOAc/CH_2Cl_2$	70	NR
4	$Rh_2(Oct)_4$	CH_2Cl_2	rt	NR
5	$Rh_2(Oct)_4$	CH_2Cl_2	70	NR
6	$Rh_2(TFA)_4$	CH_2Cl_2	rt	NR
7	$Rh_2(TFA)_4$	CH_2Cl_2	70	50
8	$[Rh_2(OAc)_2(MeCN)]_2[BF_4]_2$	CH_2Cl_2	80	94

*All reactions were run at 0.1 M in a Schlenk tube under a N_2 atmosphere for 24 h.

delight, this provided **2a** in 94% isolated yield with no trace of the other three possible cyclization products observable by ^1H NMR.

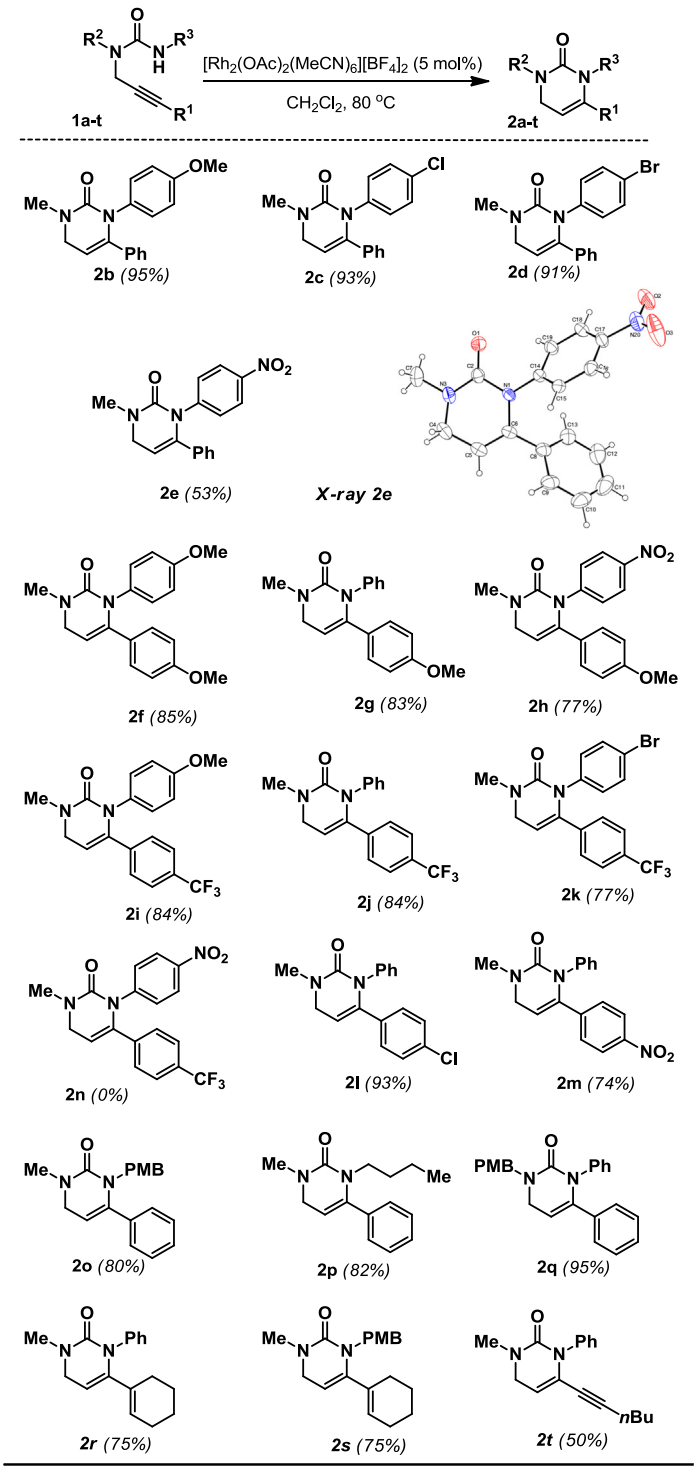
As mentioned, the competency of the more Lewis-acidic Rh(II) catalysts suggests that the propargyl ureas are inherently less nucleophilic than the related guanidines. Therefore it is likely that the nucleophilicity of the urea nitrogen and the electrophilicity of the metal–alkyne complex, might be critically coupled to the success of this reaction. Defining the limits of reactivity at these positions became the emphasis of our study on substrate scope (Table 2). With the phenyl substituted alkyne (R^1=Ph), cyclization is productive with urea substituents that span the electronic spectrum from *p*-MeOPh to *p*-NO_2Ph (e.g., **2a**–**e**). The *p*-NO_2–Ph substituted urea **2e** proved to be a highly crystalline solid, from which we were able to unambiguously prove the connectivity of these dihydropyrimidones via X-ray crystallography. Electron rich alkynes (e.g., *p*-MeOPh substituted) also reacted well with electron rich and poor ureas, **2f**–**h**. If the alkyne is electron poor (e.g., *p*-CF_3Ph substituted) it reacts well with most ureas (**2i**–**k**) but fails to react with an electron poor *p*-NO_2Ph substituted urea (e.g., **2n**). Other electron poor alkynes can also react as long as the urea is not extremely electron poor (**2l**–**m**). The reaction is also successful if the substrate is N^1,N^3-dialkyl substituted (**2o**,**p**) or N^1,N^3-alkyl/aryl substituted (**2q**). Alkyl substituted alkynes, while reactive gave complex mixtures that were intractable on a preparative scale. However, ene-ynes and yne-ynes react cleanly to give (**2r**–**t**), which are logical precursors to alkyl substitution patterns. Substrates bearing an N^3-Tosyl or N^3-Boc group are also not reactive, further defining that highly electron-withdrawing groups shut down the reaction. It is noteworthy that all of the successful cyclizations resulted in exclusive formation of the dihydropyrimidone.

When contemplating this unique reactivity, it is quite remarkable that Rh(II) catalyzes this bond forming event with two-fold thermodynamic selectivity: first to generate the 6-*endo* product and secondly to generate the C–N bond. One potential explanation for this is the Markovnikov-selective hydration of the alkyne followed by condensation of the urea on the resultant ketone. However, the addition of water (up to 1 equiv) or desiccants did not affect the efficiency of the reaction. Further, Rh(II) does not appear to be a competent Lewis acid for the condensation of a preformed urea–ketone.[13] Examples of alkyne activation by dirhodium(II) complex are quite rare.[14] Thus further investigations are warranted to understand this unique selectivity, and the potential reversibility of the initial amino- or oxo-rhodation.

2.2. Application of the resultant dihydropyrimidones

A testament to the value of these scaffolds, we identified compound **2k** capable of inhibiting proliferation of the LN-229 glioblastoma cell line (IC_{50}=25 μM) (Fig. 2). The EGF-dependent proliferation of glioblastoma cells has been directly linked to the activation of Adenosine diphosphate-ribosylation factor 6 (ARF6).[15] Known inhibitors of this enzyme, e.g., secin-44, are comprised of a 1,2-disubstituted triazole, which shares the diaryl-orientation as delivered by the methodology described above.[16] One drawback with the triazole inhibitors is their poor solubility. We reasoned

Table 2
Reaction scope



that replacement of the triazole backbone with a dihydropyrimidine should reduce the amount of unsaturation, increase polarity and thus increase solubility. To test this we prepared the analog **4** bearing the secin sidechains, via reduction of the nitro group in **2h** with Ni_2B followed EDCI mediated coupling to 5-bromofuroic acid. Indeed compound **4** inhibits marked activation of ARF6. As shown in Fig. 2B, activation of ARF6 occurs only in the presence of ARNO (ARF nucleotide-binding site opener) (lanes 1 and 2).[17] Addition of **4** inhibits this activation by nearly 50% at 50 μM (lane 3), suggesting that this small molecule might be useful for inhibiting cellular behaviors that are controlled by activated ARF6. We further investigated whether compound **4** can inhibit cell proliferation, and it does so in a dose dependent manner with an IC_{50} of 17 μM (Fig. 2C, all measurements after 48 h).[18] This activity is similar to secin-44, which has an IC_{50} of 15 μM and contrasts with an inactive variant secin-44neg (in which an acetyl group replaces

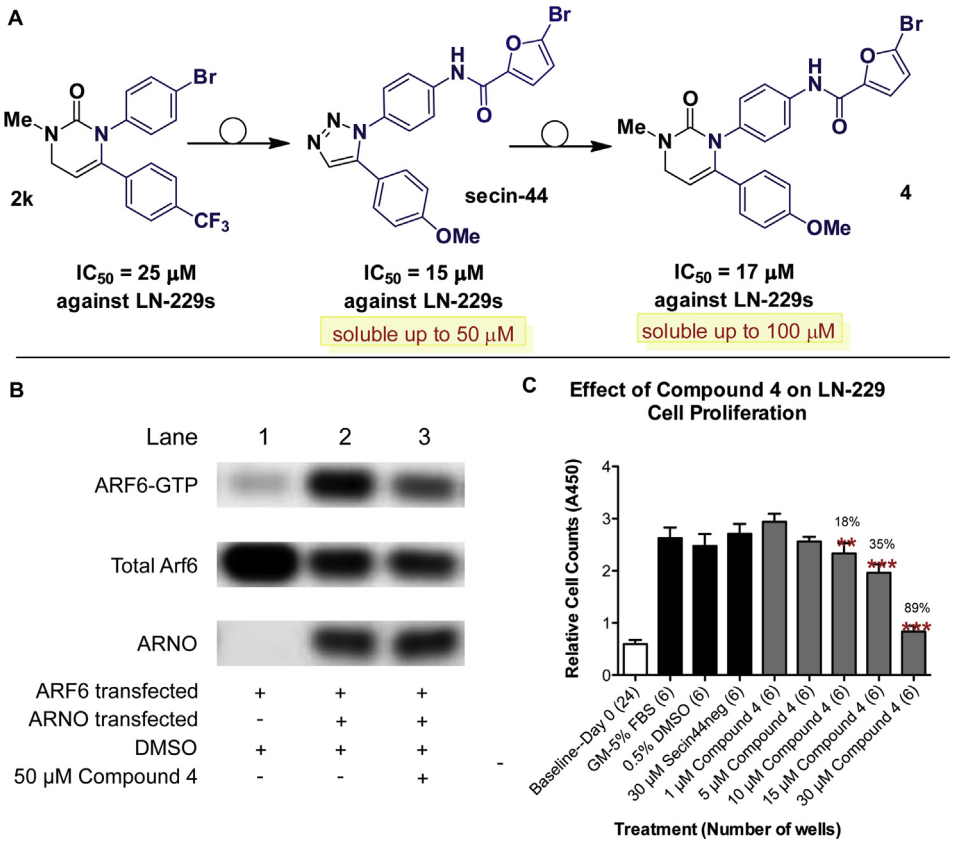


Fig. 2. Manipulation of the secin scaffold for increased solubility.

the bromofuranoic acid appendage). Moreover, **4** had improved solubility (soluble up to 100 μM) compared to secin-44, which will enable us to better evaluate the inhibition of ARF6 activation as a therapeutic target in vivo.

3. Conclusions

In conclusion, we have demonstrated that cationic dirhodium(II) is capable of catalyzing the chemo- and regioselective hydroamination of propargyl ureas. The ability of Rh(II) to selectively catalyze the 6-*endo* selective cyclization through the urea nitrogen further illustrates its unique ability to activate alkynes for nucleophilic addition. This methodology delivers dihydropyrimidones with as of yet unexplored substitution patterns, in high yield and as single regioisomers. These new structures have found use in cascade reactions and as nucleotide exchange factor inhibitors.

4. Experimental section

4.1. General

All reactions requiring anhydrous condition were conducted in flame-dried glassware under a positive pressure of nitrogen. Commercially available reagents were used as received; otherwise, materials were purified according to *Purification of Laboratory Chemicals*.[19] Dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) were degassed with nitrogen and passed through a solvent purification system (Innovative Technologies Pure Soly). Triethylamine (Et_3N) was distilled from CaH_2 immediately prior to use. Reactions were monitored by TLC and visualized by a dual short wave/long wave UV lamp and stained with $KMnO_4$. Flash chromatography was performed on Merck silica gel Kieselgel 60 (230–400 mesh) from EM science with the indicated HPLC grade solvents.

H NMR spectra were recorded on Varian Unity-300 MHz, Inova-400 MHz, or VXR-500 MHz as indicated. The chemical shifts (δ) of proton resonances are reported relative to $CHCl_3$ using the following format: chemical shift in parts per million (ppm) [multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, app=apparent), coupling constant(s) (J in Hz), integral]. ^{13}C NMR spectra were recorded at 75, 100, or 125 MHz. The chemical shifts of carbon resonances are reported relative to the deuterated solvent peak.[20,21] Infrared spectra were obtained using a Thermo Nicolet 380-FT IR spectrometer fitted with a SmartOrbit sample system. All absorptions are reported in cm^{-1} relative to polystyrene. Mass spectra were determined by ESI/APCI-TOF for HRMS in the University of Utah mass spectrometry facility.

4.2. Substrate preparation: synthesis of propargyl ureas

Given the focus of this manuscript, details for the preparation and spectral characterization of the substrate propargyl ureas corresponding to **1a**–**t** are given in Supplementary data section.

4.3. Cationic Rh(II)-catalyzed hydroamination of propargyl ureas

*4.3.1. Representative procedure for the synthesis of **2a**–**t**: 3-methyl-1,6-diphenyl-3,4-dihydropyrimidin-2(1H)-one (**2a**).* $[Rh_2(OAc)_2(CH_3CN)_6][BF_4]_2$ (16.6 mg, 0.022 mmol, 5 mol %), 1-methyl-3-phenyl-1-(3-phenylprop-2-ynyl)urea (**1a**, 116 mg, 0.44 mmol, 1.0 equiv), and CH_2Cl_2 (4.5 mL) were mixed in a flame-dried Schlenk tube under N_2. The reaction mixture was stirred at 80 °C overnight. The resulting

solution was purified through the flash column chromatography (silica gel, hexane/EtOAc=6:1 to 1:1) to give the product **2a** (109 mg, 94%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.14–7.00 (m, 10H), 5.13 (t, J=4.0 Hz, 1H), 4.09 (d, J=4.0 Hz, 2H), 3.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.6, 141.7, 139.4, 136.0, 128.9, 127.8, 127.8, 127.7, 127.6, 125.9, 100.9, 48.0, 35.3; IR (neat) 3853, 3744, 3648, 3060, 2923, 2360, 2338, 1700, 1676, 1489, 1280, 908, 727, 693, 623 cm^{-1}; HRMS (ESI) calculated for $C_{17}H_{16}N_2ONa$ m/z (M+Na) 287.1160, obsd 287.1159.

*4.3.2. 1-(4-Methoxyphenyl)-3-methyl-6-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2b**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.10–7.02 (m, 7H), 6.65 (dd, J=2.0, 6.0 Hz, 2H), 5.06 (t, J=4.0 Hz, 1H), 4.08 (d, J=4.0 Hz, 2H), 3.67 (s, 3H), 2.99 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.4, 154.9, 141.9, 136.2, 132.4, 130.1, 128.0, 127.7, 127.6, 113.2, 100.3, 55.2, 48.1, 35.4; IR (neat) 2932, 2835, 2359, 2241, 1675, 1654, 1607, 1509, 1493, 1239, 724 cm^{-1}; HRMS (ESI) calculated for $C_{18}H_{19}N_2O_2$ m/z (M+H) 295.1447, obsd 295.1447.

*4.3.3. 1-(4-Chlorophenyl)-3-methyl-6-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2c**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.14–7.06 (m, 9H), 5.15 (t, J=4.0 Hz, 1H), 4.09 (d, J=4.0 Hz, 1H), 3.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.4, 141.4, 138.1, 135.7, 131.4, 130.1, 128.0, 127.9, 127.8, 101.6, 18.0, 35.4; IR (neat) 3853, 3743, 3688, 3058, 2926, 2360, 2340, 1680, 1576, 1489, 1282, 1091, 746 cm^{-1}; HRMS (ESI) calculated for $C_{17}H_{16}N_2OCl$ m/z (M+H) 299.0951, obsd 299.0958.

*4.3.4. 1-(4-Bromophenyl)-6-phenyl-3-methyl-3,4-dihydropyrimidin-2(1H)-one (**2d**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25–7.00 (m, 9H), 5.16 (t, J=4.0 Hz, 1H), 4.08 (d, J=4.0 Hz, 1H), 3.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.3, 141.3, 138.6, 135.7, 130.9, 130.4, 128.0, 127.9, 127.8, 119.4, 101.7, 47.9, 35.4; IR (neat) 2923, 2361, 2338, 1677, 1664, 1484, 1249, 915, 868, 822, 744 cm^{-1}; HRMS (ESI) calculated for $C_{17}H_{15}N_2ONaBr$ m/z (M+Na) 365.0265, obsd 365.0265.

*4.3.5. 3-Methyl-1-(4-nitrophenyl)-6-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2e**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.99–7.97 (m, 2H), 7.35–7.33 (m, 2H); 7.14–7.07 (m, 5H), 5.31 (t, J=4.0 Hz, 1H), 4.10 (d, J=4.0 Hz, 1H), 3.3 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.4, 145.6, 144.7, 140.8, 135.2, 128.6, 128.3, 128.2, 127.4, 123.1, 103.9, 47.7, 35.5; IR (neat) 2927, 2245, 1946, 1682, 1592, 1516, 1493, 1403, 1342, 1247, 1112, 854, 754, 590 cm^{-1}; HRMS (ESI) calculated for $C_{17}H_{16}N_3O_3$ m/z (M+Na) 310.1192, obsd 310.1193.

*4.3.6. 1,6-Bis(4-methoxyphenyl)-3-methyl-3,4-dihydropyrimidin-2(1H)-one (**2f**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.02–7.00 (m, 4H), 6.66–6.61 (m, 4H), 5.00 (t, J=4.0 Hz, 1H), 4.04 (d, J=4.0 Hz, 2H), 3.68 (s, 3H), 3.67 (s, 3H), 2.97 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.7, 157.3, 155.0, 141.7, 132.4, 130.0, 129.2, 128.6, 113.1, 113.0, 99.4, 55.1, 54.9, 48.0, 35.3; IR (neat) 2933, 2836, 2361, 2338, 2241, 1675, 1652, 1608, 1509, 1437, 1360, 1290, 1241, 1174, 1109, 1031, 907, 827, 723, 592 cm^{-1}; HRMS (ESI) calculated for $C_{19}H_{21}N_2O_3$ m/z (M+H) 325.1552 obsd 325.1558.

*4.3.7. 6-(4-Methoxyphenyl)-3-methyl-1-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2g**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.14–6.61 (m, 9H), 5.07 (t, J=4.0 Hz, 1H), 4.06 (d, J=4.0 Hz, 2H), 3.68 (s, 3H), 2.99 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.8, 154.7, 131.4, 139.5, 129.1, 129.0, 128.5, 127.8, 125.8, 113.1, 100.2, 55.0, 48.0, 35.4; IR (neat) 2932, 2836, 2360, 2242, 1658, 1608, 1511, 1487, 1431, 1401, 1356, 1289, 1243, 1174, 1028, 908, 865, 761, 724, 696, 592 cm^{-1}; HRMS (ESI) calculated for $C_{18}H_{19}N_2O_2$ m/z (M+H) 295.1447, obsd 295.1448.

*4.3.8. 6-(4-Methoxyphenyl)-3-methyl-1-(4-nitrophenyl)-3,4-dihydropyrimidin-2(1H)-one (**2h**).* ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (dd, J=2.4, 4.2 Hz, 2H), 7.34 (dd, J=2.4, 7.2 Hz, 2H), 7.00 (dd, J=2.4, 6.9 Hz, 2H), 6.66 (dd, J=2.1, 6.6 Hz, 2H), 5.27 (t, J=4.0 Hz, 1H), 4.08 (d, J=4.0 Hz, 2H), 3.71 (s, 3H), 3.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.3, 154.0, 145.7, 144.7, 140.6, 128.75, 128.73, 127.6, 123.2, 102.8, 55.1, 47.6, 35.5; IR (neat) 2926, 2838, 1680, 1606, 1593, 1511, 1488, 1434, 1403, 1338, 1292, 1246, 1176, 1111, 1070, 1030, 877, 843, 757, 696, 589 cm^{-1}; HRMS (ESI) calculated for $C_{18}H_{18}N_3O_4$ m/z (M+H) 340.1297, obsd 340.1298.

*4.3.9. 1-(4-Methoxyphenyl)-3-methyl-6-(4-(trifluoromethyl)phenyl)-3,4-dihydropyrimidin-2(1H)-one (**2i**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39 (d, J=8.0 Hz, 2H), 7.24 (d, J=7.5 Hz, 2H), 7.05 (dd, J=1.5, 7.0 Hz, 2H), 6.69 (dd, J=6.5, 7.0 Hz, 2H), 5.15 (t, J=4.0 Hz, 1H), 4.12 (J=4.0 Hz, 2H), 3.70 (s, 3H), 3.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.6, 154.7, 140.8, 139.7, 132.0, 129.8, 128.1, 124.8, 124.77, 124.74, 124.71, 113.4, 101.8, 55.2, 48.0, 35.4; IR (neat) 2936, 2839, 1712, 1671, 1608, 1510, 1441, 1410, 1322, 1246, 1165, 1110, 1066, 1018, 830, 737, 556 cm^{-1}; HRMS (ESI) calculated for $C_{19}H_{17}N_2O_2F_3Na$ m/z (M+Na) 385.1140, obsd 385.1152.

*4.3.10. 3-Methyl-1-phenyl-6-(4-(trifluoromethyl)phenyl)-3,4-dihydropyrimidin-2(1H)-one (**2j**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (d, J=8.0 Hz, 2H), 7.22 (d, J=8.5 Hz, 2H), 7.21–7.12 (m, 4H), 7.05–7.03 (m, 1H), 5.20 (t, J=4.0 Hz, 1H), 4.11 (d, J=4.5 Hz, 2H), 3.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.4, 140.7, 139.6, 139.1, 128.8, 128.6, 128.3, 128.1, 128.0, 126.2, 124.83, 124.80, 124.78, 124.74, 102.6, 48.0, 35.4; IR (neat) 2929, 2348, 2246, 1676, 1488, 1404, 1365, 1320, 1281, 1248, 1163, 1107, 1066, 1017, 908, 845, 763, 695, 601, 552 cm^{-1}; HRMS (ESI) calculated for $C_{18}H_{16}N_2OF_3$ m/z (M+H) 333.1215, obsd 333.1223.

*4.3.11. 1-(4-Bromophenyl)-3-methyl-6-(4-(trifluoromethyl)phenyl)-3,4-dihydropyrimidin-2(1H)-one (**2k**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40 (d, J=7.5 Hz, 2H), 7.30–7.21 (m, 4H), 7.02 (dd, J=1.5, 8.0 Hz, 2H), 5.23 (t, J=4.0 Hz, 1H), 4.10 (d, J=4.0 Hz, 2H), 3.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.1, 139.3, 138.3, 131.3, 130.2, 127.9, 125.1, 124.8, 119.6. 103.5, 47.9, 35.4; IR (neat) 2931, 2360, 2339, 1679, 1485, 1403, 1364, 1322, 1248, 1164, 1122, 1067, 1013, 846, 823, 785, 603, 551 cm^{-1}; HRMS (ESI) calculated for $C_{18}H_{14}N_2OF_3NaBr$ m/z (M+Na) 433.0139, obsd 433.0146.

*4.3.12. 6-(4-Chlorophenyl)-3-methyl-1-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2l**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.17–7.02 (m, 9H), 5.13 (t, J=4.0 Hz, 1H), 4.09 (d, J=4.0 Hz, 2H), 3.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.5, 140.8, 139.2, 134.6, 133.4, 129.1, 128.9, 128.0, 126.2, 101.5, 48.0, 35.4; IR (neat) 3234, 3050, 2922, 2049, 1677, 1660, 1591, 1488, 1432, 1398, 1278, 1244, 1105, 1088, 1013, 909, 828, 773, 754, 744, 697, 564 cm^{-1}; HRMS (ESI) calculated for $C_{17}H_{16}N_2OCl$ m/z (M+H) 299.0951, obsd 299.0949.

*4.3.13. 3-Methyl-6-(4-nitrophenyl)-1-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2m**).* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.97 (d, J=9.5 Hz, 2H), 7.29 (d, J=6.5 Hz, 1H), 7.17–7.23 (m, 4H), 7.07–7.06 (m, 1H), 5.28 (t, J=4.0 Hz, 1H), 4.14 (d, J=4.0 Hz, 2H), 3.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.2, 146.9, 142.5, 140.2, 139.0, 128.6, 128.4, 128.3, 126.5, 123.2, 103.8, 48.0, 35.5; IR (neat) 3076, 2924, 2853, 2244, 1674, 1597, 1515, 1489, 1434, 1402, 1342, 1314, 1281, 1249, 1108, 1013, 910, 852, 767, 729, 699, 561 cm^{-1}; HRMS (ESI) calculated for $C_{17}H_{15}N_3O_3$ m/z (M+H) 332.1011, obsd 332.1015.

*4.3.14. 1-(4-Methoxybenzyl)-3-methyl-6-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2o**).* ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29–7.27 (m, 2H); 7.15–7.12 (m, 2H), 6.87–6.84 (m, 2H), 6.71–6.68 (m, 2H), 4.82 (t, J=3.9 Hz, 1H), 4.56 (s, 2H), 4.92 (d, J=3.9 Hz, 1H), 3.73 (s, 3H), 2.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.3, 155.8, 141.8, 135.7, 131.4, 128.4, 128.2, 128.1, 113.5, 100.2, 55.1, 47.8, 46.7, 35.4; IR (neat) 2932, 2834, 2360, 2339, 1652, 1611, 1510, 1494, 1442, 1242, 1174, 1031, 815,

727, 700, 645, 568 cm^{-1}; HRMS (ESI) calculated for $C_{19}H_{20}N_2O_2Na$ *m*/*z* (M+Na) 331.1422, obsd 331.1431.

*4.3.15. 1-Butyl-3-methyl-6-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2p**).* ^1H NMR ($CDCl_3$, 500 MHz) δ 7.35–7.24 (m, 5H), 4.82 (t, *J*=4.0 Hz, 1H), 4.10 (d, *J*=4.0 Hz, 1H), 3.45–3.42 (m, 2H), 2.94 (s, 3H), 1.31–1.29 (m, 2H), 1.10–1.06 (m, 2H), 0.71 (t, *J*=7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.9, 141.9, 135.9, 128.1, 128.0, 99.9, 99.8, 47.7, 43.7, 35.2, 31.5, 19.5, 13.6; IR (neat) 2955, 2928, 2870, 2360, 2338, 1652, 1493, 1431, 1400, 1275, 1083, 765, 700 cm^{-1}; HRMS (ESI) calculated for $C_{15}H_{21}N_2O$ *m*/*z* (M+H) 245.1654, obsd 245.1652.

*4.3.16. 3-(4-Methoxybenzyl)-1,6-diphenyl-3,4-dihydropyrimidin-2(1H)-one (**2q**).* ^1H NMR ($CDCl_3$, 500 MHz) δ 7.50 (dd, *J*=2.5, 7.0 Hz, 2H), 7.43–7.19 (m, 10H), 7.04 (dd, *J*=2.5, 7.0 Hz, 2H), 5.26 (t, *J*=4.0 Hz, 1H), 4.74 (s, 2H), 4.14 (d, *J*=4.0 Hz, 2H), 3.98 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.0, 154.5, 141.4, 139.5, 135.9, 129.7, 129.3, 128.9, 128.7, 128.6, 128.5, 127.8, 127.6, 127.5, 126.6, 125.9, 113.9, 113.8, 101.3, 55.1, 50.8, 45.0; IR (neat) 2928, 2834, 2173, 2065, 1709, 1660, 1512, 1445, 1283, 1246, 1174, 1110, 1032, 816, 757, 696, 614, 566 cm^{-1}; HRMS (ESI) calculated for $C_{24}H_{23}N_2O_2$ *m*/*z* (M+H) 371.1760, obsd 371.1763.

*4.3.17. 6-Cyclohexenyl-3-methyl-1-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2r**).* ^1H NMR ($CDCl_3$, 500 MHz) δ 7.27–7.14 (m, 5H), 5.73 (d, *J*=4.0 Hz, 2H), 4.89 (t, *J*=4.0 Hz, 1H), 3.94 (d, *J*=4.0 Hz, 1H), 2.93 (s, 3H), 2.03–1.86 (m, 2H), 1.59–1.57 (m, 2H), 1.32–1.25 (m, 2H), 1.21–1.16 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.6, 144.5, 139.4, 134.4, 129.4, 129.1, 128.9, 127.5, 126.0, 97.7, 47.9, 35.3, 27.4, 25.1, 22.1, 21.6; IR (neat) 3330, 3058, 2929, 2857, 1708, 1649, 1596, 1489, 1435, 1396, 1339, 1265, 1104, 1072, 920, 833, 802, 755, 731, 695, 641, 576 cm^{-1}; HRMS (ESI) calculated for $C_{17}H_{21}N_2O$ *m*/*z* (M+H) 269.1654, obsd 269.1654.

*4.3.18. 6-Cyclohexenyl-1-(4-methoxybenzyl)-3-methyl-3,4-dihydropyrimidin-2(1H)-one (**2s**).* ^1H NMR ($CDCl_3$, 500 MHz) δ 7.13 (d, *J*=9.0 Hz, 2H), 6.80 (dd, *J*=2.0, 7.0 Hz, 2H), 5.65–5.64 (m, 1H), 4.68 (t, *J*=4.0 Hz, 1H), 4.57 (s, 2H), 3.78 (d, *J*=4.0 Hz, 2H), 3.76 (s, 3H), 2.87 (s, 3H), 2.04–2.02 (m, 2H), 1.92–1.91 (m, 2H), 1.56–1.55 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.3, 155.9, 144.0, 133.6, 132.0, 128.8, 128.3, 113.5, 97.1, 55.1, 47.5, 46.6, 35.1, 28.4, 25.0, 22.3, 21.7; IR (neat) 2931, 2835, 2243, 1646, 1511, 1440, 1403, 1379, 1289, 1244, 1174, 1035, 905, 818, 724, 645, 570 cm^{-1}; HRMS (ESI) calculated for $C_{19}H_{25}N_2O_2$ *m*/*z* (M+H) 313.1916, obsd 313.1911.

*4.3.19. 6-(Hex-1-ynyl)-3-methyl-1-phenyl-3,4-dihydropyrimidin-2(1H)-one (**2t**).* ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.32 (m, 2H), 7.28–7.24 (m, 1H), 7.25–7.22 (m, 2H), 5.19 (t, *J*=4.0 Hz, 1H), 4.02 (d, *J*=4.0 Hz, 2H), 2.93 (s, 3H), 2.00 (t, *J*=6.8 Hz, 1H), 1.14–1.09 (m, 2H), 1.05–0.99 (m, 2H), 0.74 (t, *J*=7.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.6, 139.4, 129.6, 128.1, 127.2, 125.1, 103.3, 94.4, 74.4, 48.6, 35.1, 29.8, 21.4, 18.5, 13.4; IR (neat) 2955, 2928, 2870, 2360, 2229, 1668, 1597, 1488, 1433, 1325, 1284, 1250, 1101, 1070, 1023, 913, 760, 695, 567 cm^{-1}; HRMS (ESI) calculated for $C_{17}H_{21}N_2O$ *m*/*z* (M+H) 269.1654, obsd 269.1652.

4.4. Synthesis of the secin-44 analogue (4)

$N_2H_4 \cdot H_2O$ (101.0 mg) was added to a reflux mixture of nickel boride[7] (12.8 mg), 6-(4-methoxyphenyl)-3-methyl-1-(4-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one (**2h**) (16.1 mg, 0.05 mmol) in absolute EtOH (4.0 mL) and stirred at reflux for 30 min. Then, the solution was cooled to room temperature, filtered through a short pad of Celite. The filtrate was concentrated to give the aniline, which was used for the coupling reaction without further purification.

Under N_2, DCM (2.0 mL) was added to a mixture of 5-bromofuran-2-carboxylic acid (21.0 mg, 0.11 mmol), EDCI (96 mg, 0.5 mmol), DMAP (15.3 mg, 0.125 mmol), and 1-(4-aminophenyl)-6-(4-methoxyphenyl)-3-methyl-3,4-dihydropyrimidin-2(1*H*)-one (16.0 mg, 0.05 mmol). The mixture was stirred at room temperature overnight until the aniline had been consumed as judged by TLC analysis. The mixture was diluted with EtOAc (5 mL), washed with $NaHCO_3$ (aq), HCl (1 M), $NaHCO_3$ (aq), brine, and dried over $MgSO_4$. After filtration, the solution was concentrated and the residue was purified through preparative TLC (silica gel, EtOAc/hexane=3:1) to give the coupling product (**4**, 13.0 mg, 50%). ^1H NMR ($CDCl_3$, 500 MHz) δ 7.91 (s, 1H), 7.44–7.42 (m, 2H), 7.12–7.10 (m, 2H), 7.02–7.00 (m, 2H), 6.64–6.62 (m, 2H), 6.46 (d, *J*=4.0 Hz, 1H), 5.06 (t, *J*=4.0 Hz, 1H), 4.06 (d, *J*=4.0 Hz, 2H), 3.69 (s, 3H), 2.99 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.0, 154.8, 154.6, 149.3, 141.4, 136.0, 134.9, 129.6, 129.2, 128.4, 124.7, 119.2, 117.4, 114.6, 113.2, 100.2, 55.0, 48.0, 35.4; IR (neat) 3303, 2930, 2359, 2341, 1654; 1602, 1511, 1463, 1405, 1357, 1295, 1248, 1174, 1144, 1108, 1012, 952, 925, 854, 833, 798, 735, 668 cm^{-1}; HRMS (ESI) calculated for $C_{23}H_{20}N_3O_4NaBr$ *m*/*z* (M+Na) 504.0535, obsd 504.0553.

4.5. Arf6 pull-down experiments

4.5.1. Plasmid DNA construction. The ARF6 and ARNO coding sequences were amplified by PCR and then ligate into pcDNA3.1/myc-His A (Invitrogen) in fusion with Myc and a C-terminal His Tag.

4.5.2. Active ARF6 pull-down. The plasmid DNA was transfected into HEK293T cell using Lipofectamine 2000 (Invitrogen) when cell density is around 90%. The medium was changed to DMEM+1% FBS at 24 h post transfection and incubated overnight. The compound was diluted with DMEM+1% FBS according to 1:200 to get final concentration of 50 μM or 100 μM in 0.5% DMSO. Transfected cell was treated with compound or DMSO only for 4 h before pull-down. The pull-down was performed using active Arf6 pull-down and detection kit from Thermo Scientific (cat#: 26186).

4.6. Solubility determinations

Solubility determinations were made by adding a 10 mM solution of secin-44 or compound **4** in DMSO to pull-down medium (DMEM+1% FBS) to final concentrations of 1, 10, 50, 100 and 200 μM. After standing overnight the solutions were observed under a microscope and the concentration at which the compound began crystallizing was noted as its maximum solubility.

Acknowledgements

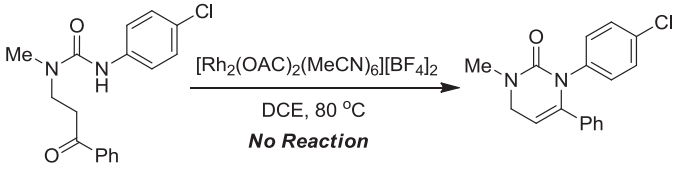
We are thankful to Dr. Jim Muller for help with mass spectral analysis. R.E.L. is grateful to the National Institutes of Health, General Medical Sciences (R01-GM090082 and P41-GM08915) for financial support of this research. D.Y.L. is supported by grants from the National Institutes of Health, NCI (R01HL066277) and the Department of Defense (W81XWH0910260).

Supplementary data

Analytical data for all new compounds including ^1H and ^{13}C NMR spectra. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2013.04.071.

References and notes

1. (a) Kappe, C. O. *Eur. J. Med. Chem.* **2000**, *35*, 1043; (b) Singh, K.; Arora, D.; Singh, K.; Singh, S. *Mini-Rev. Med. Chem.* **2009**, *9*, 95; (c) Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937.
2. (a) Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J. *Science* **1999**, *286*, 971; (b) Atwal, K. S.; Swanson, B. N.; Unger, S. E.; Floyd, D. M.; Moreland, S.; Hedberg, A.; O'Reilly, B. C. *J. Med. Chem.* **1991**, *34*, 806.
3. Biginelli, P. *Gazz. Chim. Ital.* **1893**, *23*, 360.
4. For excellent reviews see: (a) Kappe, C. O. *J. Org. Chem.* **1997**, *62*, 7201; (b) Kappe, C. O. *Acc. Chem. Res.* **2000**, *33*, 879; (c) Wan, J.-P.; Liu, Y. *Synthesis* **2010**, *23*, 3943.
5. For enantioselective variants see: (a) Li, N.; Chen, X. _H.; Song, J.; Luo, S.-W.; Fan, W.; Gong, L.-Z. *J. Am. Chem. Soc.* **2009**, *131*, 15301; (b) Gong, L.-Z.; Chen, X.-H.; Xu, X.-Y. *Chem.—Eur. J.* **2007**, *13*, 8920; (c) Huang, Y.; Yang, F.; Zhu, C. *J. Am. Chem. Soc.* **2005**, *127*, 16386; (d) Lou, S.; Taoka, B. M.; Ting, A.; Schaus, S. E. *J. Am. Chem. Soc.* **2005**, *127*, 11256; (e) Goss, J. M.; Schaus, S. E. *J. Org. Chem.* **2008**, *73*, 7651; (f) Chen, X.-H.; Xu, X.-Y.; Liu, H.; Cun, L.-F.; Gong, L.-Z. *J. Am. Chem. Soc.* **2006**, *128*, 14802.
6. Oberg, K. M.; Rovis, T. *J. Am. Chem. Soc.* **2011**, *133*, 4785.
7. Giles, R. L.; Sullivan, J. D.; Steiner, A. M.; Looper, R. E. *Angew. Chem., Int. Ed.* **2009**, *48*, 3116; (b) Gainer, M. J.; Bennett, N. R.; Takahashi, Y.; Looper, R. E. *Angew. Chem., Int. Ed.* **2011**, *50*, 684.
8. Gold catalyzed intramolecular annulation of substituted aminoalkynes: (a) Verniest, G.; Padwa, A. *Org. Lett.* **2008**, *10*, 4379; (b) Hashmi, A. S. K. *Chem. Rev.* **2007**, *107*, 3180; (c) Istrate, F. M.; Buzas, A. K.; Jurberg, I. D.; Odabachian, Y.; Gagosz, F. *Org. Lett.* **2008**, *10*, 925; (d) Hirano, K.; Inaba, Y.; Watanabe, T.; Oishi, S.; Fujii, N.; Ohno, H. *Adv. Synth. Catal.* **2010**, *352*, 368; (e) Perl, N. R.; Ide, N. D.; Prajapati, S.; Perfect, H. H.; Duron, S. G.; Gin, D. Y. *J. Am. Chem. Soc.* **2010**, *132*, 1802; (f) Ye, D.; Zhang, X.; Zhou, Y.; Zhang, D.; Zhang, L.; Wang, H.; Jiang, H.; Liu, H. *Adv. Synth. Catal.* **2009**, *351*, 2770; (g) Enomoto, T.; Girard, A.-L.; Yasui, Y.; Takemoto, Y. *J. Org. Chem.* **2009**, *74*, 9158; (h) Enomoto, T.; Obika, S.; Yasui, Y.; Takemoto, Y. *Synlett* **2008**, 1647; (i) Hashmi, A. S. K.; Rudolph, M.; Schymura, S.; Visus, J.; Frey, W. *Eur. J. Org. Chem.* **2006**, 4905; (j) Kang, J.-E.; Kim, H.-B.; Lee, J.-W.; Shin, S. *Org. Lett.* **2006**, *8*, 3537; (k) Hashmi, A. S. K.; Weyrauch, J. P.; Frey, W.; Bats, J. W. *Org. Lett.* **2004**, *6*, 4391; (l) Milton, M. D.; Inada, Y.; Nishibayashi, Y.; Uemura, S. *Chem. Commun.* **2004**, 2712; (m) Ritter, S.; Horino, Y.; Lex, J.; Schmalz, H.-G. *Synlett* **2006**, 3309; (n) Verniest, G.; England, D.; De Kimpe, N.; Padwa, A. *Tetrahedron* **2010**, *66*, 1496; (o) Campbell, M. J.; Toste, F. D. *Chem. Sci.* **2011**, *2*, 1369.
9. Silver: (a) Ermolat'ev, D. S.; Mehta, V. P.; Van der Eycken, E. V. *Synlett* **2007**, 3117; (b) Yamada, W.; Sugawara, Y.; Cheng, H. M.; Ikeno, T.; Yamada, T. *Eur. J. Org. Chem.* **2007**, 2604; (c) Patil, N. T.; Pahadi, N. K.; Yamamoto, Y. *J. Org. Chem.* **2005**, *70*, 10096; (d) Peng, A. Y.; Ding, Y. X. *Org. Lett.* **2005**, *7*, 3299; Ag(I)-catalyzed N-cyclization: (e) Peshkov, V. A.; Pereshivko, O. P.; Sharma, S.; Meganathan, T.; Parmar, V. S.; Ermolat'ev, D. S.; Van der Eycken, E. V. *J. Org. Chem.* **2011**, *76*, 5867; (f) Huang, Q.; Hunter, J. A.; Larock, R. C. *Org. Lett.* **2001**, *3*, 2973; (g) Asao, N.; Yudha, S.; Nogami, T.; Yamamoto, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 5526; (h) Su, S.; Porco, J. A., Jr. *J. Am. Chem. Soc.* **2007**, *129*, 7744; (i) Yu, X.; Yang, X.; Wu, J. *Org. Biomol. Chem.* **2009**, *7*, 4526; (j) Ghavtadze, N.; Fröhlich, R.; Würthwein, E.-U. *Eur. J. Org. Chem.* **2010**, 1787; (k) Chen, Z.; Yu, X.; Wu, J. *Chem. Commun.* **2010**, 6356; (l) Chen, Z.; Wu, J. *Org. Lett.* **2010**, *12*, 4856.
10. Palladium: (a) Majumdar, K. C.; De, Mirupam; Roy, B. *Synthesis* **2010**, 4207; (b) Narsireddy, M.; Yamamoto, Y. *J. Org. Chem.* **2008**, *73*, 9698; (c) Patil, N. T.; Wu, H.; Yamamoto, Y. *J. Org. Chem.* **2007**, *72*, 6577; (d) Patil, N. T.; Lutete, L. M.; Wu, H.; Pahadi, N. K.; Gridnev, I. D.; Yamamoto, Y. *J. Org. Chem.* **2006**, *71*, 4270; (e) Bajracharya, G. B.; Huo, Z.; Yamamoto, Y. *J. Org. Chem.* **2005**, *70*, 4883; (f) Lutete, L. M.; Kadota, I.; Yamamoto, Y. *J. Am. Chem. Soc.* **2004**, *126*, 1622; (g) Saito, A.; Iimura, K.; Hanzawa, Y. *Tetrahedron Lett.* **2010**, *51*, 1471; (h) Bacchi, A.; Chiusoli, G. P.; Costa, M.; Sani, C.; Gabriele, B.; Salerno, G. *J. Organomet. Chem.* **1998**, *562*, 35; (i) Bacchi, A.; Costa, M.; Gabriele, B.; Pelizzi, G.; Salerno, G. *J. Org. Chem.* **2002**, *67*, 4450; (j) Gabriele, B.; Plastina, P.; Salerno, G.; Raffaella Mancuso, R.; Costa, M. *Org. Lett.* **2007**, *9*, 3319.
11. Copper: (a) Krasnova, L. B.; Hein, J. E.; Fokin, V. V. *J. Org. Chem.* **2010**, *75*, 8662; (b) Kimura, M.; Kure, S.; Yoshida, Z.; Tanaka, S.; Fugami, K.; Tamaru, Y. *Tetrahedron Lett.* **1990**, *31*, 4887; (c) Ohe, K.; Ishihara, T.; Chatani, N.; Kawasaki, Y.; Murai, S. *J. Org. Chem.* **1991**, *56*, 2267.
12. Rempel, G. A.; Legzdins, P.; Smith, H.; Wilkinson, G. *Inorg. Synth.* **1972**, *13*, 87; Crawford, C. A.; Matonic, J. H.; Streib, W. E.; Huffman, J. C.; Dunbar, K. R.; Christou, G. *Inorg. Chem.* **1993**, *32*, 3125.
13. We prepared the urea–ketone, which failed to cyclize under the reaction conditions. Similar substrates are known to give moderate to low yields of dihydropyrimidones in the presence of mineral acid catalysts, see: Xue, N.; Lu, X.; Hu, Y. *J. Heterocycl. Chem.* **2008**, *45*, 1095.



14. (a) Ota, K.; Chatani, N. *Chem. Commun.* **2008**, 2906; (b) Ota, K.; Lee, S. I.; Tang, J.-M.; Takachi, M.; Nakai, H.; Morimoto, T.; Sakurai, H.; Kataoka, K.; Chatani, N. *J. Am. Chem. Soc.* **2009**, *131*, 15203; (c) Lee, S. I.; Chatani, N. *Chem. Commun.* **2009**, 371; (d) Shikanai, D.; Murase, H.; Hata, T.; Urabe, H. *J. Am. Chem. Soc.* **2009**, *131*, 3166; (e) Miki, K.; Washitake, Y.; Ohe, K.; Uemura, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 1857; (f) Moniz, G. A.; Wood, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 5095.
15. Li, M.; Wang, J.; Ng, S. S. M.; Chan, C.; He, M.; Yu, F.; Lai, L.; Shi, C.; Chem, Y.; Yew, T.; Kung, H.; Lin, M. C. *Cancer* **2009**, *115*, 4959.
16. Stumpfe, D.; Bill, A.; Novak, N.; Loch, G.; Blockus, H.; Geppert, H.; Becker, T.; Schmitz, A.; Hoch, M.; Kolanus, W.; Famulok, M.; Bajorath, J. *ACS Chem. Biol.* **2010**, *5*, 839.
17. For leading references see: (a) Donaldson, J. G.; Klausner, R. D. *Curr. Opin. Cell Biol.* **1994**, *6*, 527; (b) Frank, S.; Upender, S.; Hansen, S. H.; Cassanova, J. E. *J. Biol. Chem.* **1998**, *273*, 23.
18. See Supplementary data for more detailed protocols and statistical analysis.
19. Armarego, W. L. F.; Chai, C. L. L. *Purification of Laboratory Chemicals*, 7th ed.; Elsevier: Oxford, UK, 2013.
20. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512.
21. Hoye, T. R.; Hansen, P. R.; Vyvyan, J. R. *J. Org. Chem.* **1994**, *59*, 4096.