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Synthesis and Evaluation of a Novel E-ring Modified α-Hydroxy Keto Ether Analogue of Camptothecin

Wu Du,[a] Dennis P. Curran,[a,*] Robert L. Bevins,[b] Stephen G. Zimmer,[b] Junhong Zhang[c] and Thomas G. Burke[b,c,*]

[a]*University of Pittsburgh, Department of Chemistry, Pittsburgh, PA 15260, USA*
[b]*Colleges of Pharmacy Medicine, and Experimental Therapeutics Program, Markey Cancer Center, University of Kentucky, Lexington, KY 40506, USA*
[c]*Tigen Pharmaceuticals, Inc., Lexington, KY 40506, USA*

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Abstract—The synthesis of a novel E-ring modified keto ether analogue of camptothecin and homocamptothecin by the cascade radical annulation route is reported. The analogue, Du1441, is an isomer of homocamptothecin, but includes the α-hydroxy carbonyl functionality that camptothecin possesses and homocamptothecin lacks. Despite these similarities, the new keto ether analogue is inactive in cell assays, and implications for the structure/activity relationship are discussed. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Isolated from the Chinese tree *Camptotheca acumunata* by Wani and Wall in 1966,[1,2] camptothecin **1** is the parent of a large and important family of anti-tumor agents. Topotecan and irinotecan are analogs of camptothecin that are now being marketed in several countries, and a number of other analogs are the subject of ongoing clinical or preclinical evaluation.[3–6]

Camptothecin (CPT) and its relatives are topoisomerase I poisons (Fig. 1). Topoisomerase I catalyzes DNA relaxation by a mechanism that involves transient, enzyme-linked single strand breaks. Camptothecin is thought to interfere with this reaction by blocking the rejoining step, and this results in an accumulation of a reversible Topo I-CPT-DNA ternary complex (termed the cleavable complex). Prolonged exposure of cancer cells to compounds in the camptothecin class results in irreversible DNA damage and cell death.[5] The structure of the cleavable complex is not known with certainty, but two binding models have been proposed by Pommier[7] and Hol[8] and their coworkers.

Although there are differences, both models postulate that a crucial feature for the six-membered α-hydroxy lactone E-ring of camptothecin is the formation of intermolecular hydrogen bonds with amino acid residues and a nucleotide at the enzyme–DNA complex binding site. Thus, the six-membered α-hydroxy lactone E-ring is important for activity. At the same time, the E-ring lactone is a critical structural liability due to its sensitivity to hydrolysis. Camptothecin opens rapidly to an inactive hydroxy acid form in human plasma, with only negligible active lactone form remaining at equilibrium.[9] The chemical instability caused by the α-hydroxy lactone may account for the failure of otherwise active compounds in the camptothecin series in treating tumors successfully. Accordingly, the development of E-ring modified camptothecin analogs with improved chemical stability while maintaining binding ability to the enzyme–DNA complex is an important goal.

Over the years, a number of E-ring modified camptothecin analogs have been prepared. These include camptothecin lactol[10] and lactam derivatives,[11] a ring opened hydroxy amide,[12] an α-halolactone,[1,13] an α-azide lactone,[13] an α-aminolactone,[13,14] and an α-exo-methylene lactone.[15] Because all these compounds were either inactive or showed significantly decreased activity in cell assays relative to camptothecin, it was gradually accepted that E-ring modifications were uni-

*Corresponding authors. Tel.: +1-412-624-8240; fax: +1-412-624-9861; e-mail: curran@pitt.edu (D. P. Curran), tetlett@pitt.edu (T. G. Burke)

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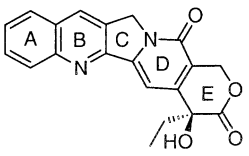
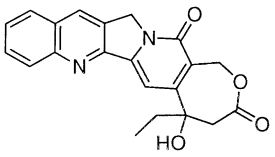


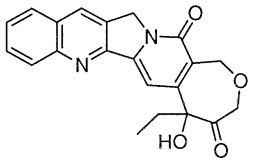
1

camptothecin
(an α-hydroxy lactone)



2

homocamptothecin
(a β-hydroxy lactone)



3

α-hydroxy ketoether analog

Figure 1.

versally detrimental. In an important breakthrough, Lavergne and coworkers found that homocamptothecin **2**, a camptothecin analog with an expanded seven-membered β-hydroxy lactone, has improved stability and retains anti-tumor activity.[16] A number of A-ring functionalized 7-silylhomocamptothecin (homosilatecan) analogs show promise as anti-tumor agents.[17] Although the expanded β-hydroxy lactone of homocamptothecin is chemically much different from the α-hydroxy lactone of camptothecin, it is still subject to opening under physiological conditions. Nonetheless, the discovery of homocamptothecin **2** shows that fruitful modification of the E-ring of camptothecin is possible and raises anew the question of whether there are any non-lactone analogs of camptothecin that retain biological activity.

In this paper, we report the synthesis and study of a novel α-hydroxy keto ether E-ring analog **3** of camptothecin and homocamptothecin. This keto ether is an isomer of homocamptothecin with the extra E-ring methylene group and the carbonyl group reversed. Unlike both camptothecin and homocamptothecin, it lacks a lactone. Like homocamptothecin it has a seven membered ring, but like camptothecin it has the α-hydroxy carbonyl combination (which homocamptothecin lacks). Despite these similarities, keto ether **3** is inactive. An isomeric keto ether side product was also produced in the synthesis, and this was also tested and found to be inactive. The implications of these results within the current understanding of the structure/activity relationship of camptothecin are briefly analyzed.

Results and Discussion

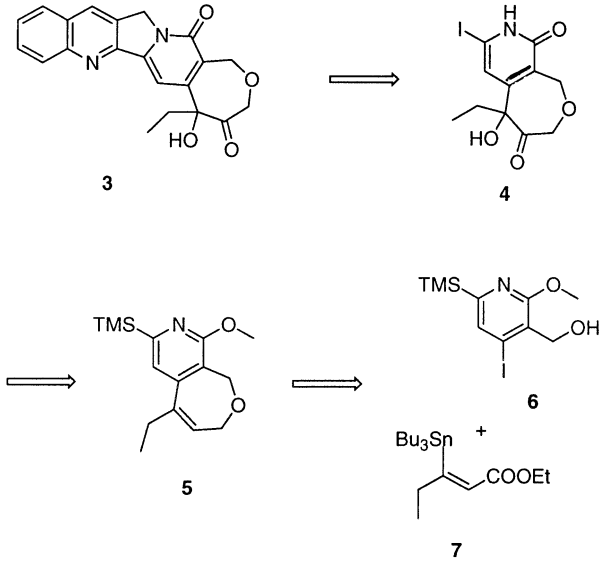
Synthesis of the ketoethers

The retrosynthetic plan is based on a cascade radical annulation[18a,b] (Scheme 1), and calls for a propargylated iodopyridone **4** and phenylisonitrile. This approach has been used to generate camptothecin and many known and new analogs, including DB-67, which is currently undergoing preclinical development.[18c] Iodopyridone **4** with an α-hydroxy ketoether functionality can be derived from cyclic ether **5** by dihydroxylation and subsequent oxidation. The cyclic ether can be obtained by Stille coupling from the stannyl ester **7** and a pyridine **6**, both of which are known.[18,19]

The first stage of the synthesis is shown in Scheme 2. Stannylcupration of alkyne **8** provided the known tin reagent **7**.[19] Pyridine **6** (Scheme 1), an early intermediate in the camptothecin synthesis,[18] was protected with the MOM (methoxymethyl) group to give pyridine **9**. Then CuCl accelerated Stille coupling[20] between **7** and **9** gave the Z-ester **10** in 94% yield. Reduction of the ester **10** with LAH yielded the Z-allylic alcohol **11** in 95% yield. The treatment of alcohol **11** with TFA was expected to remove the MOM group and accomplish etherification in one pot.[21] However, the reaction of **11** with TFA gave bis-trifluoroacetate **12** instead. Basic hydrolysis of ester **12** yielded diol **13** ready for cyclization.

Several attempted cyclizations of diol **13** (Mitsunobu,[22] $ZnCl_2$[23]) were not successful; however, the desired cyclization was smoothly accomplished by BuLi/tosyl chloride method.[24] Diol **13** was first treated with 1 equiv of BuLi followed by 1 equiv of tosyl chloride. This presumably gives a monotosylate, which was not characterized. The second equivalent of BuLi was directly added to effect the desired etherification. After workup and flash chromatography, pure ether **5** was isolated in 81% yield. The S_N2' reaction product, ether **14** with a five-membered ether ring, was also observed. Ethers **5** and **14** were formed in 10:1 ratio as determined by NMR.

Conversion of **5** to the radical precursor **4** is shown in Scheme 3. Dihydroxylation was performed on ether **5** in the absence of the chiral ligand[18] to give racemic diol **15**



Scheme 1.

in 50% yield. Oxidation of 1,2-diols to α-hydroxyketones requires a very mild oxidant to avoid cleavage of the diol C–C bond to give a ketone aldehyde. IBX, the precursor of Dess–Martin periodinane, was reported to be a mild oxidant to convert alcohols to aldehydes[25] and to convert 1,2-diols to α-hydroxyketones.[26] Thus, IBX was prepared using reported procedures,[27] and reacted with diol **15** in DMSO. This reaction gave the desired α-hydroxy ketoether **16** in quantitative yield. Subsequently, the TMS group was replaced with iodide by treatment of the ketoether **16** with the solution of ICl in dichloromethane. Besides the desired product **18**, the reaction also gave unreacted starting **16** and a chlorinated product **17** which was not observed before using solid ICl.[18] These three products could be separated by careful chromatography. Finally, the methoxy group in ketone **18** was removed with TMSI (generated in situ from TMSCl and NaI) to give iodopyridone **4** in 87% yield.

The *N*-propargylation of iodopyridone **4** using the well-developed conditions[18d] surprisingly gave two products, compound **19** in 37% yield and compound **20** in 31% yield (Scheme 4). These two compounds are isomers as shown by high resolution MS, and have similar functionalities as shown by NMR and IR spectroscopy. The presence of ketone in both compounds was verified by the peak around 210 ppm in the ^{13}C NMR spectrum and the absorption at 1720 cm^{-1} in the IR spectrum. Through extensive HMBC and HMQC NMR experiments, we were able to assign the structures as the expected product **19** (37%) and a rearranged product **20** (31%).

The rearranged product **20** presumably results from an anionic 1,2 shift, as shown in Scheme 5.

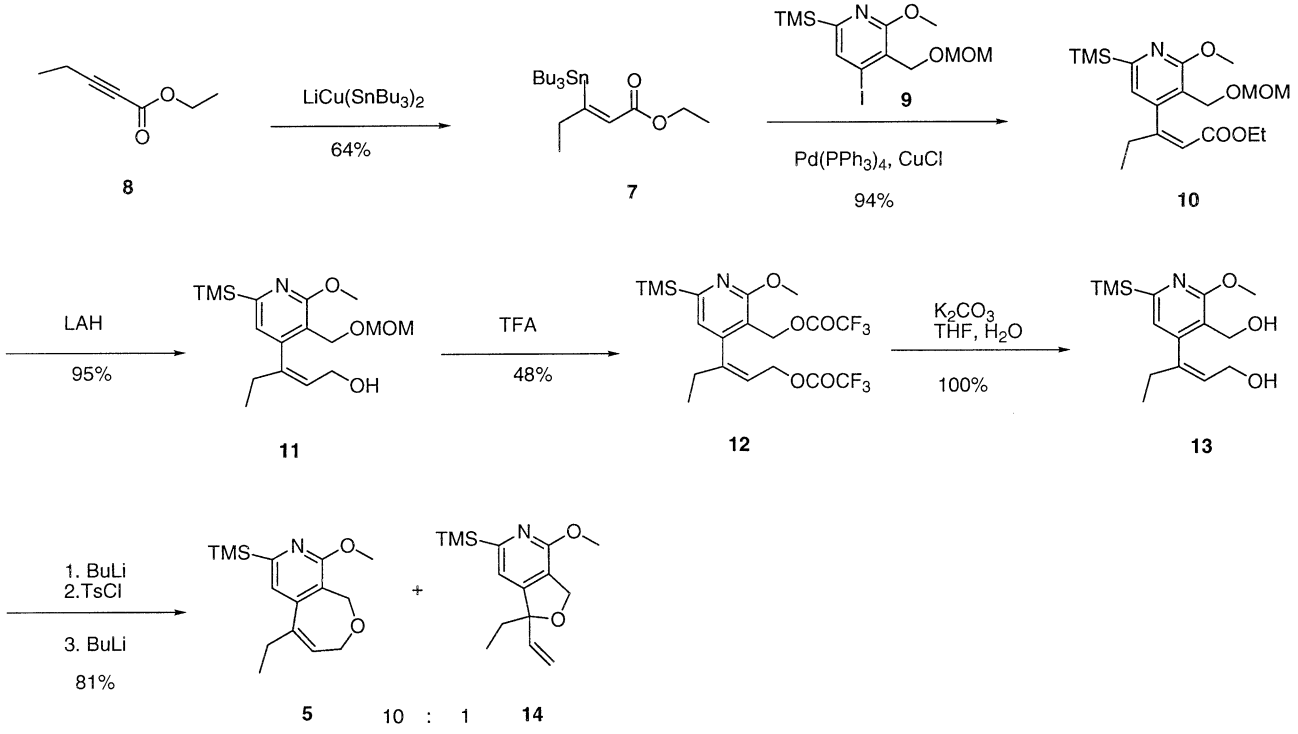
Compounds **19** and **20** were individually subjected to the cascade radical annulation, as shown in Scheme 6. Irradiation of **19** and **20** with phenylisonitrile in the presence of hexamethylditin in benzene with sunlamp generated the desired α-hydroxy ketoether analogue Du1441 **3** and the rearranged α-hydroxy ketoether analogue Du1442 **21**. Both new products were isolated as yellow solids in 43% yield after purification.

HPLC analysis

Using HPLC with fluorescence detection, the purity of both Du1441 **3** and Du1442 **21** samples were observed to be in excess of 98% pure. Unlike camptothecin, which readily hydrolyzes to form its ring-opened carboxylate form in PBS (greater than 85% of camptothecin converted to its carboxylate form in PBS, pH 7.4 after 3 h of incubation), HPLC analysis confirmed the expected solution stability for Du1441 and Du1442. There was no evidence of hydrolysis of the two compounds over 3 h incubation in PBS, pH 7.4, at 37 °C.

Cellular assays

In cytotoxicity assays employing MDA-MB-435 S human breast cancer cells, camptothecin was found to exhibit an IC_{50} value of 15 nM. In marked contrast to the high potency of the parent agent, no indication of cytotoxic activity was observed for either Du1441 and



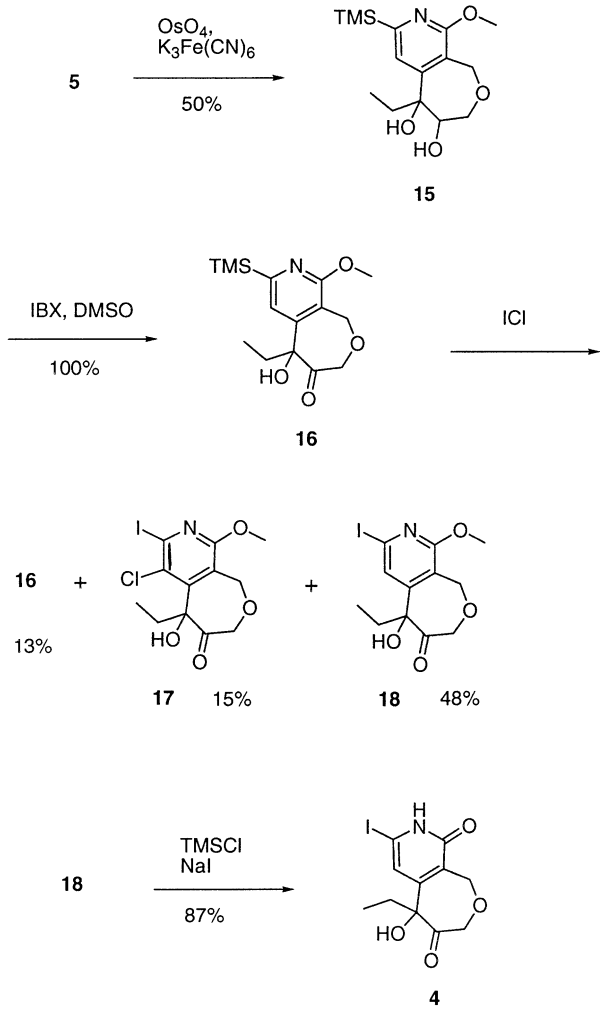
Scheme 2.

Du1442 analogues even at concentrations as high as 1 μM. Thus, while the structural changes created non-lactone camptothecins resistant to hydrolysis under near physiological conditions of pH, ionic strength and temperature, the compounds were found to be devoid of anticancer activity.

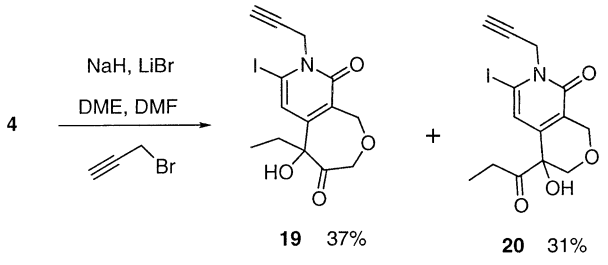
Conclusions

The cascade radical annulation approach has recently been used to make many analogues of camptothecin and homocamptothecin, but the current work shows that its utility extends significantly beyond that. Two novel non-lactone E-ring analogues of camptothecin, **3** and **21**, have been prepared. Their preparation requires only the synthesis of an appropriately substituted iodopydridone, **19** and **20**, and then the final two steps of the synthesis proceed as projected. The approach should be amenable to making many other kinds of non-lactone E-ring analogues of camptothecin.

The rearranged analogue **21** is quite different from camptothecin, so its inactivity is not surprising. However, the ketoether **3** is very similar to both camptothecin and homocamptothecin, so its lack of activity is striking. This raises anew the question of whether lactone opening must occur for camptothecins to bind to the topo I/DNA complex. This question is currently being addressed through biological and computational approaches. Results suggest that the lactone ring is not opened in the cleavable complex, so the quest for non-lactone analogues of camptothecin should continue.



Scheme 3.

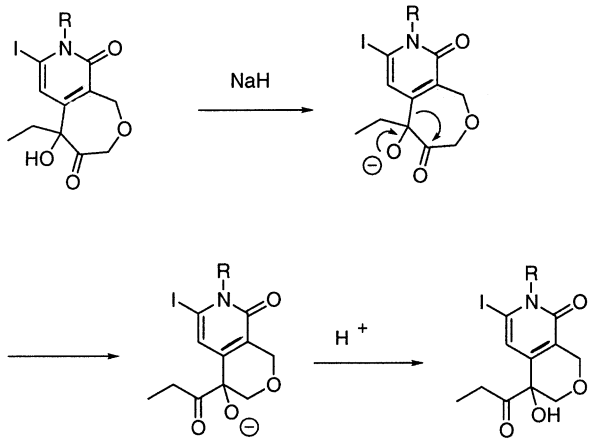


Scheme 4.

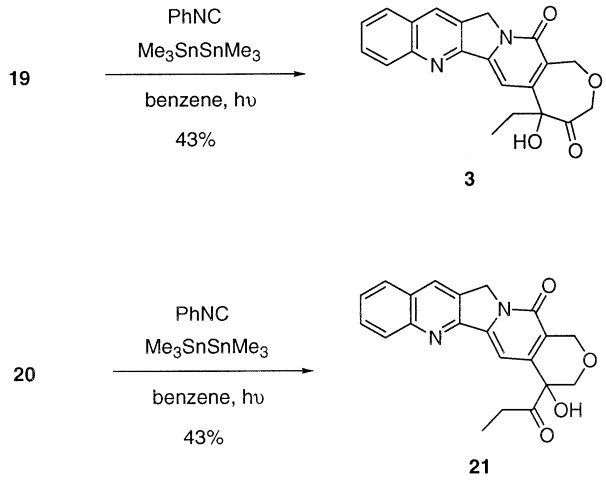
Experimental

General

All reactions were run under argon unless otherwise noted. THF, benzene were freshly distilled from



Scheme 5.



Scheme 6.

sodium/benzophenone. LiCl was dried by heating to 120 °C under vacuum overnight. Other reagents were used as they were received from Aldrich.

***(Z)*-3-[2-Methoxy-3-methoxymethoxymethyl-6-(trimethylsilyl)pyridin-4-yl]pent-2-enoic acid ethyl ester (10).** To a flame dried flask charged with Ar was added dry LiCl (1.5 g, 36 mmol), tetrakis(triphenylphosphine)palladium (800 mg, 0.7 mmol) and CuCl (3.0 g, 30 mmol). This mixture was degassed under high vacuum with an Ar purge. Then anhydrous DMSO (20 mL) was added with stirring, followed by the solution of compound **9** (2.4 g, 6.3 mmol) in DMSO (5 mL) and a solution of compound **7** (3.0 g, 7.2 mmol) in DMSO (5 mL). The resulting mixture was degassed by freeze–thaw procedure under Ar, then stirred at room temperature for 30 min and then heated to 60 ^{0}C for 20 h. The mixture was then cooled, diluted with EtOAc and washed with a mixture of brine and 5% aqueous NH_4OH. The aqueous layer was back washed with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated to give a crude residue which was purified by flash chromatography using 10% EtOAc in hexane as eluent to give 2.13 g of the title compound as a colorless oil in 94% yield: IR 2948, 1725, 1645, 1576, 1555, 1452, 1341, 1247, 1187, 1045, 919, 840, 755; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.26 (s, 9H), 1.00 (t, J=7.1 Hz, 3H), 1.12 (t, J=7.4 Hz, 3H), 2.44 (m, 2H), 3.40 (s, 3H), 3.95 (m, 2H), 3.99 (s, 3H), 4.36 (d, J=10.4 Hz, 1H), 4.51 (d, J=10.4 Hz, 1H), 4.64 (d, J=6.6 Hz, 1H). 4.69 (d, J=6.6 Hz, 1H), 5.92 (t, J=1.5 Hz, 1H), 6.73 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −2.0, 11.4, 13.7, 33.0, 53.2, 55.1, 59.7, 61.8, 96.4, 114.7, 117.0, 120.7, 150.3, 158.2, 161.7, 164.1, 165.3; HRMS m/z calcd for $C_{19}H_{31}NO_5Si$ 381.1972, found 381.1975; LRMS (EI) m/z 381 (M^+) 366, 336, 319, 304, 290, 278, 262, 246, 232.

***(Z)*-3-[2-Methoxy-3-methoxymethoxymethyl-6-(trimethylsilyl)pyridin-4-yl]pent-2-en-1-ol (11).** A solution of compound **10** (2.1 g, 5.5 mmol) in anhydrous ether (30 mL) was cooled to 0 °C, then $LiAlH_4$ (220 mg, 5.8 mmol) was added. The resulting suspension was allowed to warm to room temperature. After 5 h, another portion of $LiAlH_4$ (220 mg, 5.8 mmol) was added and the reaction was stirred for 5 h more. The reaction mixture was then slowly (cautious, exothermic) poured into ice cold saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and concentrated to give a crude product which was further purified by flash chromatography (30% EtOAc in hexane) to give the title compound (1.87 g) as a white solid in 95% yield: IR 3416, 2950, 1576, 1553, 1451, 1341, 1247, 1151, 1101, 1044, 920, 841, 755, 697; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.27 (s, 9H), 1.04 (t, J=7.4 Hz, 3H), 2.24 (q, J=7.3 Hz, 2H), 3.43 (s, 3H), 3.69 (m, 2H), 4.00 (s, 3H), 4.31 (d, J=9.8 Hz, 1H), 4.65 (d, J=9.8 Hz, 1H), 4.68 (d, J=6.2 Hz, 1H), 4.80 (d, J=6.2 Hz, 1H), 5.83 (t, J=7.6 Hz, 1H), 6.75 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −1.97, 11.9, 31.6, 53.3, 55.6, 59.2, 61.5, 96.5, 115.8, 122.7, 126.2, 142.8, 150.1, 162.1, 165.0; HRMS m/z calcd for $C_{17}H_{29}NO_4Si$ 339.1866, found 339.1852; LRMS (EI) m/z 339 (M^+), 308, 277, 262, 248.

Trifluoroacetic acid *Z*-4-(1-ethyl-3-trifluoroacetoxy-propenyl)-2-methoxy-6-(trimethylsilyl)pyridin-3-yl-methyl ester (12). Compound **11** (1.9 g, 5.6 mmol) was dissolved in TFA (100 mL) and the mixture was stirred at room temperature for 24 h. The pink solution was concentrated under vacuum, diluted with EtOAc and then washed with saturated aqueous $NaHCO_3$. The organic layer was dried and concentrated. The crude residue was then purified by flash chromatography (5% EtOAc in hexane) to give 1.28 g the bistrifluoroacetate as a colorless oil in 48% yield: IR 2959, 1788, 1556, 1553, 1449, 1349, 1222, 1150, 842; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.31 (s, 9H), 1.07 (t, J=7.4 Hz, 3H), 2.33 (q, J=7.4 Hz, 2H), 3.63 (dd, J=12.0, 10.1 Hz, 1H), 4.01 (s, 3H), 4.54 (m, 2H), 5.21 (d, J=11.4 Hz, 1H), 5.33 (d, J=11.4 Hz, 1H), 5.77 (t, J=7.1 Hz, 1H), 6.81 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −2.2, 11.6, 31.8, 53.5, 62.2, 65.0, 111.7, 114.6 (qd, J=284, 7.2 Hz), 119.0, 122.0, 147.6, 149.2, 157.2 (q, J=42 Hz), 162.1, 168.0; HRMS m/z calcd for $C_{19}H_{23}NO_5F_6Si$ 487.1250, found 487.1240; LRMS (EI) m/z 487 (M^+), 472, 391. 376, 360, 277, 262, 249, 232, 218, 204.

***(Z)*-3-(3-Hydroxymethyl-2-methoxy-6-trimethylsilyl-pyridin-4-yl)-pent-2-en-1-ol (13).** To the solution of compound **12** (1.0 g, 2.05 mmol) in THF (10 mL) was added water (2 mL) and K_2CO_3 (0.5 g). The reaction was stirred for 2 h and diluted with EtOAc. The EtOAc layer was washed with water, brine and dried over $MgSO_4$. Evaporation of the solvent gave the diol **13** (740 mg) as white solid in quantitative yield: IR 3318, 2963, 1577, 1550, 1449, 1340, 1247, 1011, 840. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30 (s, 9H), 1.03 (t, J=7.4 Hz, 3H), 2.28 (m, 2H), 3.63 (dd, J=12.0, 10.1 Hz, 1H), 3.79 (dd, J=12.0, 5.5 Hz, 1H), 4.07 (s, 3H), 4.34 (d, J=11.8 Hz, 1H), 4.71 (d, J=11.8 Hz, 1H), 5.86 (dd, J=10.0, 5.7 Hz, 1H), 6.79 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −2.0, 11.7, 31.6, 53.1, 56.9, 58.6, 118.9, 122.5, 125.7, 142.9, 148.5, 161.8, 164.2; HRMS (M-H_2O and C_2H_5) m/z calcd for $C_{13}H_{18}NO_2Si$ 248.1107, found 248.1114; LRMS (EI) m/z 277 (M-H_2O), 262, 248.

5-Ethyl-1-methoxy-3-trimethylsilyl-7-H,9-H-8-oxa-2-aza-benzocycloheptene (5). BuLi (1.46 mL of 1.6 M solution in hexane, 2.34 mmol) was slowly added to the solution of compound **13** (690 mg, 2.34 mmol) in THF (50 mL) under Ar and at 0 °C. This solution was stirred at 0 °C for 30 min, then *p*-toluenesulfonyl chloride (2.34 mL of 1 M solution in THF, 2.34 mmol) was slowly added. After 1 h, another portion of BuLi (1.46 mL of 1.6 M solution in hexane, 2.34 mmol) was introduced. The reaction was gradually warmed to room temperature and stirred for 7 h, then poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was dried and concentrated to give a crude product. The ^{1}H NMR spectrum of this crude product indicated that compound **5** and **14** were formed in 10 to 1 ratio. The crude product was purified by flash chromatography (5% of EtOAc in hexane) to give the title compound (524 mg) as a colorless oil in 81% yield: IR 2962, 1724, 1580, 1551, 1453, 1346, 1246, 1132, 1047, 838; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.31 (s, 9H), 1.10 (t, J=7.4 Hz, 3H), 2.56 (m, 2H), 3.88 (d, J=6.2 Hz, 1H), 4.00 (s, 3H),

3.88 (m, 2H), 4.52 (s, 2H), 6.12 (t, $J=6.3$ Hz, 1H), 7.07 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −1.9, 13.3, 28.2, 53.2, 60.1, 63.5, 118.7, 119.0, 126.0, 146.2, 149.0, 161.2, 164.2; HRMS *m/z* calcd for $C_{15}H_{23}NO_2Si$ 277.1498, found 277.1493; LRMS (EI) *m/z* 277 (M^+), 262, 248, 234.

5-Ethyl-1-methoxy-3-trimethylsilyl-5,6,7,9-tetrahydro-8-oxa-2-aza-benzocycloheptene-5,6-diol (15). Compound **5** (600 mg, 2.17 mmol) was dissolved in a mixed solvent *t*-BuOH/H_2O (1:1, 10 mL), then to this solution was added $K_3Fe(CN)_6$ (2.0 g, 6.08 mmol), 800 mg of K_2CO_3 (800 mg, 5.8 mmol) and $CH_3SO_2NH_2$ (400 mg, 4.2 mmol) successively. This suspension was then cooled to 0 °C and an OsO_4 solution (0.3 mL of 2.5% solution in *t*-BuOH, 0.023 mmol) was added. The reaction was warmed to room temperature and stirred overnight. The reaction was quenched with saturated $NaSO_3$ and extracted with EtOAc. The organic layer was dried and concentrated, the residue was purified by flash chromatography (40% EtOAc in hexane) to give the title compound (337 mg) as a white foam in 50% yield: IR 3421 (br), 2954, 1575, 1548, 1446, 1341, 1247, 1047, 838; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.29 (s, 9H), 0.89 (t, $J=7.3$ Hz, 3H), 1.83 (m, 2H), 3.74 (d, $J=2.9$ Hz, 1H), 3.95 (s, 3H), 4.04 (d, $J=13.0$ Hz, 1H), 4.15 (dd, $J=13.1$, 3.4 Hz, 1H), 4.32 (d, $J=14.9$ Hz, 1H). 5.44 (d, $J=14.9$ Hz, 1H), 7.55 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −1.9, 7.2, 29.3, 53.4, 66.6, 72.4, 76.2, 79.9, 116.5, 122.5, 152.1, 160.3, 164.0; HRMS *m/z* calcd for $C_{15}H_{25}NO_4Si$ 311.1553, found 311.1566; LRMS (EI) *m/z* 311 (M^+), 296, 267, 250, 238.

5-Ethyl-5-hydroxy-1-methoxy-3-trimethylsilyl-5,9-dihydro-8-oxa-2-aza-benzocycloheptene-6-one (16). To a solution of diol **15** (262 mg, 0.84 mmol) in DMSO (5 mL) was added a solution of IBX (354 mg, 1.26 mmol) in DMSO (2 mL). The reaction was stirred at room temperature overnight. The cloudy mixture was then diluted with EtOAc and washed with saturated aqueous Na_2SO_3 three times and finally brine. The organic layer was dried ($MgSO_4$) and concentrated. The crude residue was purified by flash chromatography using 20% EtOAc in hexane as eluent to give the title compound (260 mg) as a white solid in quantitative yield: IR 3472, 2957, 1719, 1572, 1555, 1450, 1342, 1246, 1087, 1057, 839; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30 (s, 9H), 0.93 (t, $J=7.4$ Hz, 3H), 2.09 (m, 1H), 2.57 (m, 1H), 3.98 (s, 3H), 4.23 (d, $J=18.7$ Hz, 1H), 4.37 (s, 1H), 4.64 (d, $J=17.6$ Hz, 1H), 4.71 (d, $J=18.8$ Hz, 1H), 5.26 (d, $J=17.6$ Hz, 1H), 7.62 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −1.9, 8.4, 34.7, 53.3, 70.6, 75.7, 83.5, 116.4, 119.7, 146.3, 159.6, 163.0, 210.4; HRMS *m/z* calcd for $C_{15}H_{23}NO_4Si$ 309.1396, found 309.1401; LRMS (EI) *m/z* 309 (M^+), 294, 267, 250, 236, 260.

5-Ethyl-5-hydroxy-3-iodo-1-methoxy-5,9-dihydro-8-oxa-2-aza-benzocyclohepten-6-one (18). A solution of compound **16** (230 mg, 0.74 mmol) in tetrachloromethane (6 mL) was cooled to 0 ^{0}C, then to this solution was added ICl (3 mL of a 1 M solution in CH_2Cl_2, 3 mmol). The mixture was stirred in dark and at room temperature for 20 h. The reaction mixture was then diluted with EtOAc and washed with saturated aqueous Na_2SO_3 solution. The organic layer was dried and evaporated. The crude product was purified by flash chromatography (10% EtOAc in hexane) to give, in the order of elution, 30 mg of the starting **16**, 130 mg of the title compound as a pale yellow foam in 48% yield, and 45 mg of chlorinated product **17**: IR 3439, 2922, 1723, 1556, 1454, 1370, 1280, 1239, 1127, 1084, 963, 849; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, $J=7.4$ Hz, 3H), 2.09 (m, 1H), 2.48 (m, 1H), 3.94 (s, 3H), 4.22 (d, $J=18.8$ Hz, 1H), 4.32 (s, 1H), 4.54 (d, $J=17.6$ Hz, 1H), 4.71 (d, $J=18.8$ Hz, 1H). 5.18 (d, $J=17.6$ Hz, 1H), 7.83 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 8.3, 34.7, 54.5, 70.2, 75.8, 83.0, 111.7, 116.7, 125.5, 149.7, 159.4, 163.0, 209.6; HRMS *m/z* calcd for $C_{12}H_{14}NO_4I$ 362.9968, found 362.9962; LRMS (EI) *m/z* 363 (M^+), 321, 304, 276.

4-Chloro-5-ethyl-5-hydroxy-3-iodo-1-methoxy-5H,9H-8-oxa-2-aza-benzocycloheptен-6-one (17). IR 3443, 2953, 1725, 1555, 1456, 1357, 1285, 12411131, 1087, 1054, 960; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, $J=7.5$ Hz, 3H), 2.41 (m, 1H), 2.68 (m, 1H), 3.93 (s, 3H), 4.21 (d, $J=17.1$ Hz, 1H), 4.39 (d, $J=16.9$ Hz, 1H), 4.67 (d, $J=16.8$ Hz, 1H). 5.30 (d, $J=17.0$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 7.2, 29.3, 54.7, 68.3, 76.8, 84.7, 120.2, 122.0, 147.6, 149.5, 157.4, 209.5; HRMS (EI) *m/z* calcd for $C_{12}H_{13}NO_4ClI$ 396.9578, found 396.9587; LRMS (EI) *m/z* 397 (M^+), 355, 341, 310, 249.

5-Ethyl-5-hydroxy-3-iodo-5,9-dihydro-2*H*-8-oxa-2-aza-benzocycloheptene-1,6-dione (4). To a solution of methyl ether 18 (115 mg, 0.32 mmol) and NaI (71 mg, 0.47 mmol) in MeCN (1.5 mL) was slowly added chlorotrimethylsilane (61 μL) followed by a solution of H_2O in MeCN (160 μL). After heating in the dark at 60 °C for 5 h, the mixture was cooled and poured into 5% NaSO3 aqueous solution and brine (1:1), and extracted with EtOAc. The EtOAc layer was dried and evaporated, purified by flash chromatography (40% EtOAc in hexane) to give 105 mg of the title compound as white solid in 87% yield: IR 3452, 2935, 1719, 1620, 1590, 1532, 1125, 731; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (t, $J=7.4$ Hz, 3H), 2.12 (m, 1H), 2.49 (m, 1H), 4.25 (d, $J=18.8$ Hz, 1H), 4.37 (s, 1H), 4.56 (d, $J=18.2$ Hz, 1H), 4.71 (d, $J=18.8$ Hz, 1H). 5.25 (d, $J=18.2$ Hz, 1H), 7.30 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 8.3, 34.5, 71.1, 76.0, 82.8, 91.6, 117.2, 124.0, 150.3, 162.6, 209.4; HRMS *m/z* calcd for $C_{11}H_{12}NO_4I$ 348.9811, found 348.9811; LRMS (EI) *m/z* 349 (M^+), 307, 291, 262.

Compounds 19 and 20. Iodopyridone **4** (95 mg, 0.272 mmol) was dissolved in anhydrous DME (2 mL) and anhydrous DMF (0.7 mL) under Ar. The solution was cooled to 0 ^{0}C, and NaH (12 mg of 60% suspension in mineral oil, 0.3 mmol) was added. Bubbles (H_2) were observed. After the reaction mixture was stirred at 0 °C for 10 min, LiBr (47 mg, 0.54 mmol) was added and the reaction was stirred at room temperature for 15 min. Then, a 80% solution of propargyl bromide in toluene (121 μL, 1.1 mmol) was added to the reaction, and the mixture was heated at 65 °C in dark for 20 h. The final solution was poured into brine, and extracted with EtOAc. The EtOAc layer was dried and evaporated to

give a crude mixture which was then purified by flash chromatography (40% EtOAc in hexane) to give, in the order of elution, 37.3 mg of compound **19** as a white foam in 37% yield and 31.1 mg of compound **20** as a white foam in 31% yield.

5-Ethyl-5-hydroxy-3-iodo-2-prop-2-ynyl-5,9-dihydro-2*H*-8-oxa-2-aza-benzocycloheptene-1,6-dione (19). IR 3447, 3295, 2927, 1720, 1633, 1584, 1517, 1422, 1132, 987; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, $J=7.4$ Hz, 3H), 2.09 (m, 1H), 2.35 (t, $J=2.4$ Hz, 1H), 2.47 (m, 1H), 4.23 (d, $J=18.8$ Hz, 1H), 4.35 (s, 1H), 4.52 (d, $J=18.3$ Hz, 1H), 4.69 (d, $J=18.7$ Hz, 1H). 5.01 (dd, $J=17.0$, 2.5 Hz, 1H), 5.09 (dd, $J=17.0$, 2.4 Hz, 1H), 5.19 (d, $J=18.4$ Hz, 1H), 7.42 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 8.2, 34.3, 43.6, 71.5, 73.1, 75.8, 82.3, 96.3, 118.2, 124.9, 148.4, 159.5, 209.2; HRMS *m*/*z* calcd for $C_{14}H_{14}NO_4I$ 386.9968, found 386.9971; LRMS (EI) *m*/*z* 387 (M^+), 329, 290.

4-Hydroxy-6-iodo-4-propionyl-7-prop-2-ynyl-1,3,4,7-tetrahydropyrano[3,4-*c*]pyridin-8-one (20). IR 3421, 3292, 2925, 1712, 1645, 1527, 1422, 1199, 1110, 972; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, $J=7.1$ Hz, 3H), 2.37 (m, 1H), 2.37 (t, $J=2.1$ Hz, 1H), 2.90 (m, 1H), 3.65 (d, $J=12.0$ Hz, 1H), 3.82 (d, $J=12.0$ Hz, 1H), 4.56 (d, $J=17.2$ Hz, 1H), 4.73 (s, 1H), 4.82 (d, $J=17.2$ Hz, 1H). 5.06 (d, $J=2.0$ Hz, 2H), 6.69 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 7.3, 31.7, 43.6, 64.2, 69.6, 73.3, 73.6, 97.4, 116.7, 125.8, 145.7, 158.9, 210.2; HRMS *m*/*z* calcd for $C_{14}H_{14}NO_4I$ 386.9968, found 386.9972; LRMS (EI) *m*/*z* 387 (M^+), 331. 292.

Isohomocamptothecin Du1441 (3). A solution of compound 19 (10 mg, 0.026 mmol) in benzene (0.5 mL) was added to a NMR tube under argon. Then to this solution was added a 1 M solution of phenylisonitrile in benzene (0.1 mL, 0.1 mmol) and of hexamethylditin (15 μL). The NMR tube was sealed and irradiated with 275 W GE sunlamp for 2 h. A yellow precipitate was observed. The reaction mixture was then applied on silica gel column and first washed with dichloromethane followed by 5% acetone in CH_2Cl_2 to afford 4 mg of the title compound as a pale yellow powder in 43% yield: IR: 3352, 2927, 1719, 1652, 1586, 1428, 1320, 1246, 1130, 1072, 996, 849, 765; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (t, $J=7.4$ Hz, 3H), 2.26 (m, 1H), 2.58 (m, 1H), 4.33 (d, $J=18.7$ Hz, 1H), 4.48 (s, 1H), 4.76 (d, $J=18.4$ Hz, 1H), 4.78 (d, $J=18.7$ Hz, 1H). 5.27 (s, 2H), 5.46 (d, $J=18.4$ Hz, 1H), 7.65 (t, $J=7.3$ Hz, 1H), 7.83 (t, $J=7.3$ Hz, 1H), 7.92 (d, $J=8.2$ Hz, 1H), 7.97 (s, 1H), 8.24 (d, $J=8.2$ Hz, 1H), 8.37 (s, 1H); ^{13}C NMR ($CDCl_3$/CD_3OD 125 MHz) δ 8.4, 29.8, 34.6, 49.4, 71.9, 76.1, 100.3, 125.6, 127.8, 128.1, 128.2, 128.4, 129.7, 130.6, 131.1, 143.3, 148.9, 150.0, 153.0, 159.4, 209.8; HRMS *m*/*z* calcd for $C_{21}H_{18}N_2O_4$ 362.1267, found 362.1266, LRMS (EI) *m*/*z* 362 (M^+), 303, 289, 275, 248, 219, 205.

4-Hydroxy-4-propionyl-1,3,4,12-tetrahydro-2-oxa-6,12a-diaza-dibenzo[b,h]fluoren-13-one (21).[28] Using procedure for **3**, compound **21** (4 mg) was prepared from compound 20 in 43% yield as pale yellow powder: IR 3339, 2930, 1712, 1651, 1589, 1442, 1265, 1020, 739; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06 (t, $J=7.2$ Hz, 3H), 2.41 (m, 1H), 2.92 (m, 1H), 3.8 (d, $J=11.8$ Hz, 1H), 3.96 (d, $J=11.9$ Hz, 1H), 4.78 (d, $J=17.1$ Hz, 1H), 5.03 (d, $J=17.0$ Hz, 1H). 5.29 (s, 2H), 7.65 (t, $J=7.5$ Hz, 1H), 7.82 (t, $J=7.5$ Hz, 1H), 7.93 (d, $J=7.5$ Hz, 1H), 8.19 (d, $J=7.5$ Hz, 1H), 8.39 (s, 1H); HRMS *m*/*z* calcd for $C_{21}H_{18}N_2O_4$ 362.1267, found 362.1252; LRMS (EI) *m*/*z* 362 (M^+), 344, 306, 288, 277, 248, 219, 205.

HPLC analysis

HPLC analyses were carried out on a Waters Alliance 2690 Separations Module equipped with a Waters™ 474 Scanning fluorescence Detector. For both Du1441 and Du1442, fluorescence excitation was set at 370 nm and emission at 440 nm, gain = 10 and separations were carried out on a Waters Symmetry C_{18} 5 μm particle size reversed-phase 1503.9mm column. In the analyses of Du1441, the mobile phase consisted of 65% TEAA buffer [2% triethylamine in water (v/v), adjusted to pH 5.5 with glacial acetic acid] and 35% acetonitrile. In the analyses of Du1442, mobile phase consisted of 69% TEAA buffer and 31% acetonitrile. Flow rates of 1 mL/min were used in all experiments. Fluorescence output signal was monitored and integrated using *Millennium*[32] Chromatography Manager software.

Cytotoxicity assays

MDA-MB-435 S+ Human breast cancer cells were maintained in cell culture flasks with MEM supplemented with 10% FBS, antibiotics, and essential amino acids (Life Technologies). Cells were incubated at 37 °C using a CO_2 concentration of 5%. Sulforhodamine B (SRB) staining was used to assess cell survival following drug treatment. In these studies, 435 S+ cells were plated onto 24-well plates. Following 24 h of incubation, DMSO formulations of camptothecin, DU1441, and DU1442 were added to each well at concentrations ranging from 15 to 1000 nM. Following 72 h of treatment, cells were fixed and analyzed.

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References and Notes

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28. Due to extremely low solubility of **21** in NMR solvents, we were unable to obtain ^{13}C NMR spectrum for this compound.