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FORMATION OF THE C_3-C_4 UNSATURATED FRAMEWORK OF CRIBROSTATIN 4 *VIA* DEAD-MEDIATED OXIDATION OF AN ALLYLIC TERTIARY AMINE

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Abstract – The formal synthesis of the tetrahydroisoquinoline alkaloid cribrostatin 4 (**1**) is described. The synthesis features as a key step a DEAD-mediated oxidation of an allylic tertiary amine to an iminium ion immediately followed by a Pictet-Spengler cyclization to form the pentacyclic skeleton of cribrostatin 4.

INTRODUCTION

The tetrahydroisoquinoline family of antitumor agents comprise a clinically significant family of antitumor drugs.[1] Within this family, four main structural motifs are noteworthy. The tetrahydroisoquinolines that are represented by quinocarcin, tetrazomine, lemonomycin, and the bioxalomycins are biosynthetically derived from phenylalanine (or tyrosine) and glutamic acid. The second sub-type, which includes ecteinascidin 743 (**3**), the saframycins (**6**), jorumycin (**5**) and members of the renieramycin family (**1**, **2**), are biosynthetically derived from two tyrosine units. With respect to the chemically reactive functionality within this family, several members (quinocarcin, tetrazomine, bioxalomycins) contain a fused oxazolidine ring while others contain either a carbinolamine or an α-aminonitrile at C-21 (eg., ecteinascidin 743, lemonomycin, saframycin A). Cribrostatin 4 (**1**, also named renieramycin H) was independently isolated from the blue sponge *Cribrochalina* collected in reef passages in the Republic of Maldives by Petit and from the bright blue sponge *Haliclona cribicutis* by Parameswaran and coworkers.[2] One unique structural feature of cribrostatin 4 (**1**) and renieramycin I (**2**), is the presence of the C_3-C_4 benzylic olefinic residue (Figure 1). Seminal work by Danishefsky and coworkers led to the disclosure of the first total synthesis of cribrostatin 4 (**1**) *via* a "lynchpin Mannich"

cyclization protocol to establish the pentacyclic core.[3] The Danishefsky strategy effectively installed the C_3-C_4 benzylic olefin *via* C4-ketone reduction and dehydration.[3] As part of our long-standing interest in the chemistry and biology of the tetrahydroisoquinoline alkaloids,[1,4-9] we recently reported the asymmetric total synthesis of cribrostatin 4[5] *via* a C_3-C_4 fused tricyclic β-lactam species which served as an efficient precursor for the creation of the C_3-C_4 benzylic olefin.[6] Cribrostatin 4 displays low micromolar antimicrobial and antitumor activities compared with the most potent members of this family, such as ecteinascidin 743 (**3**), phtalascidin 650 (**4**), jorumycin (**5**) or saframycin S (**6**) that exhibit nanomolar cytotoxic activities.[1] The extraordinary activity of the latter compounds is due to the presence of a carbinolamine (or functional equivalent) moiety at C_{21} that allows the formation of a potent electrophilic iminium ion species implicated in the formation of covalent bonds with DNA.[1] Thus, we speculate that the cytotoxic activity of cribrostatin 4 (**1**) should be significantly increased by introducing a carbinolamine function at C_{21} in place of the amide carbonyl residue in the natural product. The synthesis of analogues of ecteinascidin-743, phtalascidin 650, jorumycin or saframycin S bearing a C_3-C_4 alkene might provide a valuable platform from which to explore the stereo-electronic influence of the C_3-C_4 double bond on the reactivity of the electrophilic iminium ion species as this relates to cytotoxic activity. We report herein a new method to access the desired C_3-C_4 unsaturated pentacyclic skeleton.

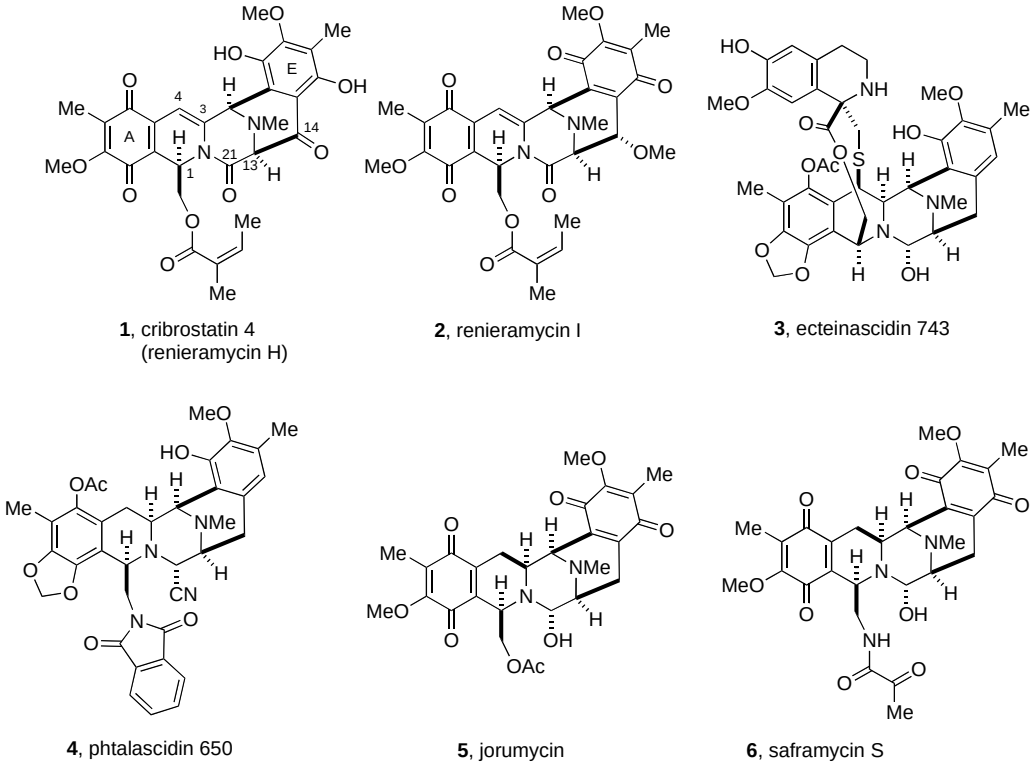
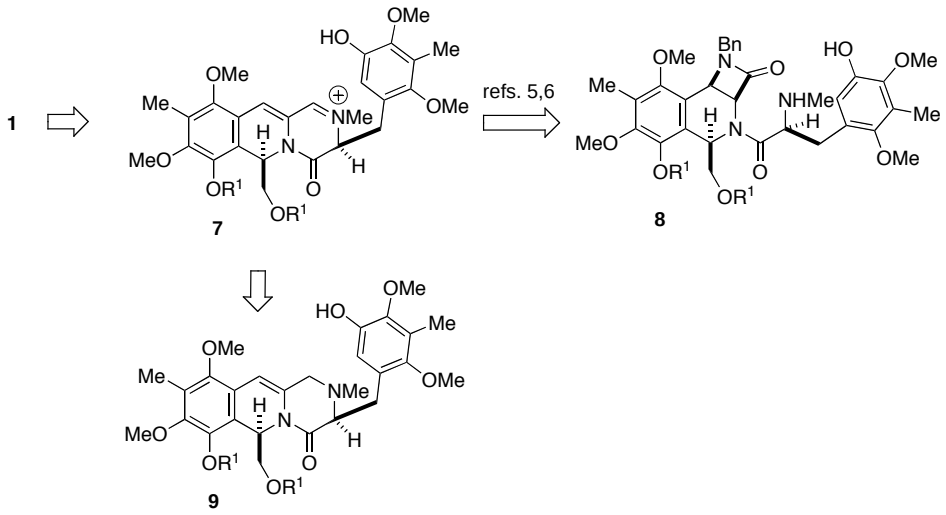


Figure 1. Tetrahydroisoquinoline alkaloids.

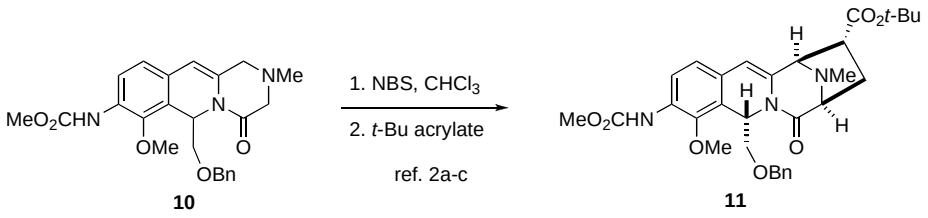
RESULTS AND DISCUSSION

Our retrosynthesis is based on the Pictet-Spengler cyclization of the conjugated iminium ion intermediate **7**.[1] Two different strategies to form the key iminium ion species can be envisioned (Scheme 1). The first strategy relied on the reductive opening/elimination of the C_3-C_4 β-lactam in **8** immediately followed by spontaneous iminium ion formation and cyclization to the pentacyclic framework.[6] As mentioned above, this new conceptual approach to tetrahydroisoquinolines was recently successfully conscripted for the asymmetric total synthesis of cribrostatin 4.[5]



Scheme 1. Retrosynthesis of Cribrostatin 4.

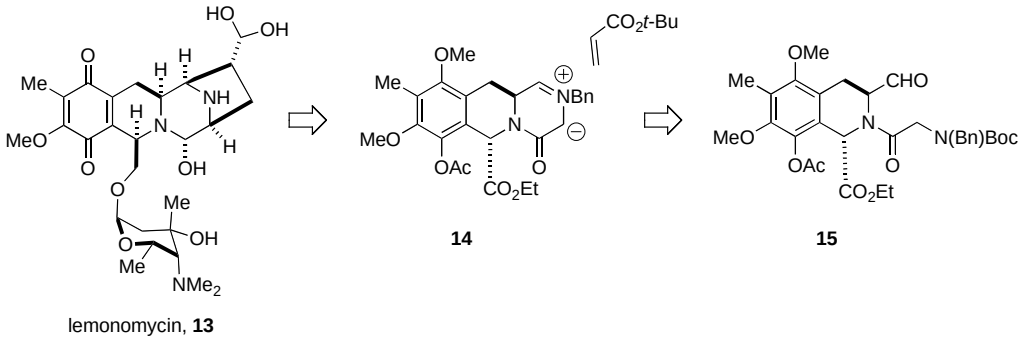
Our second option was envisioned to exploit the direct oxidation of the allylic tertiary amine **9** into the common iminium ion species **7**. This approach was inspired by our NBS-mediated oxidation of structurally related allylic tertiary amines to iminium intermediates that were then converted into azomethine ylides that underwent [1,3]-dipolar cycloadditions with acrylates, culminating with the total syntheses of tetrazomine and quinocarcinamide (Scheme 2).[4a-c]



Scheme 2. NBS-mediated oxidation of tertiary allylic amines.

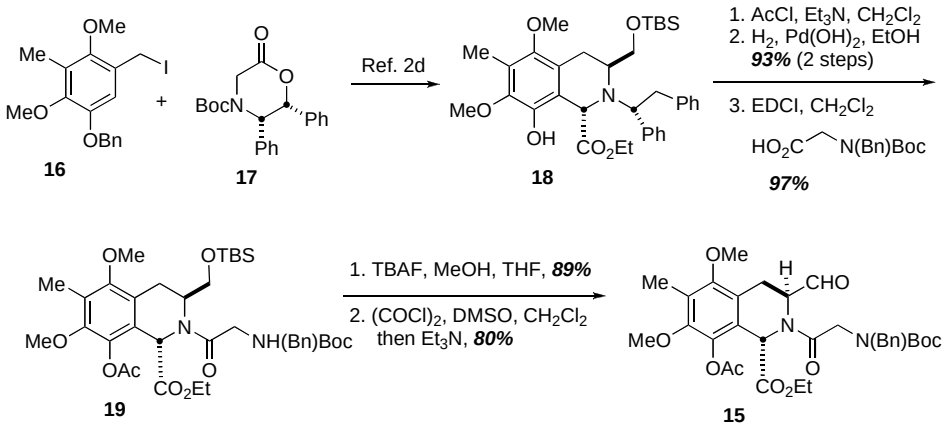
Moreover, we have observed an interesting air-mediated autoxidation of an allylic tertiary amine in the course of synthetic studies towards lemonomycin, another member of the tetrahydrisoquinoline family of

antitumor antibiotics.[10-12] Our retrosynthesis of lemonomycin (**13**) required a [1,3]-dipolar cycloaddition between *t*-butyl acrylate and azomethine ylide **14**. The latter would be the result of the condensation of the amine and aldehyde functions in **15** after *N*-*t*-Boc removal, followed by deprotonation of the resulting iminium ion intermediate (Scheme 3).



Scheme 3. Retrosynthesis of Lemonomycin.

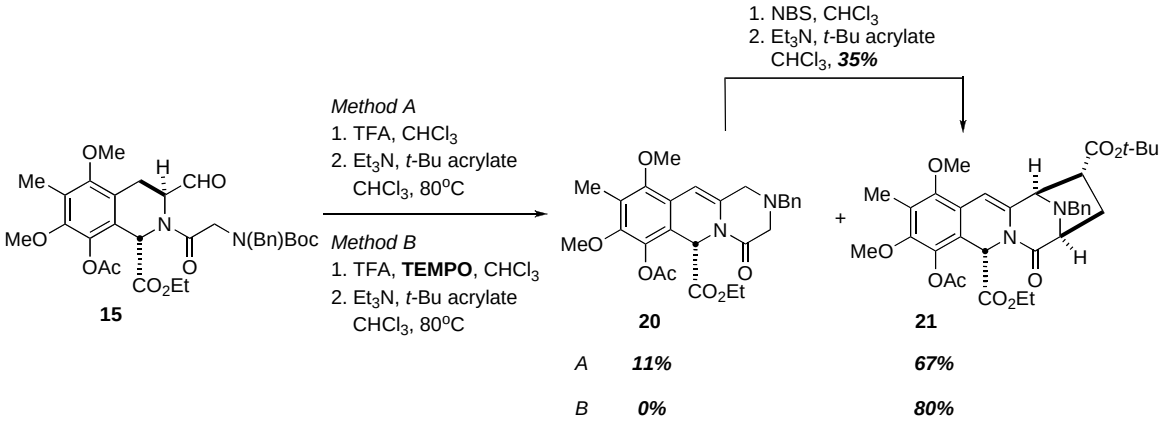
Tetrahydroisoquinoline **18** was produced from the coupling product of the chiral glycine template **17** and benzyl iodide **16** according to our previously reported protocol.[13] Tetrahydroisoquinoline **18** was transformed to the *N*-*t*-Boc amino aldehyde **15** in five steps as described in Scheme 4.



Scheme 4. Synthesis of amino aldehyde **15**.

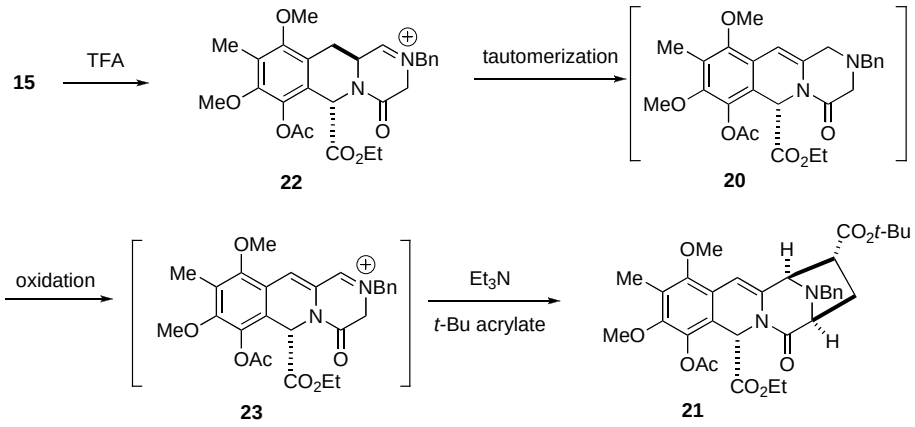
Interestingly and unexpectedly, *N*-*t*-Boc removal from **15** with TFA followed by treatment with triethylamine and *t*-butyl acrylate did not produce the expected cycloadduct but afforded the allylic tertiary amine **20** (11%) and the C_3-C_4 unsaturated tetracycle cycloadduct **21** in 67% yield (Scheme 5). We were able to selectively obtain the unsaturated cycloadduct **21** (80%) by adding TEMPO to promote the oxidation of the allylic amine during the *N*-*t*-Boc removal step. Moreover, treatment of allylic amine

20 with NBS, followed by addition of triethylamine and *t*-butyl acrylate led to the formation of the unsaturated cycloadduct **21** in 35% yield.



Scheme 5. Unexpected formation of the unsaturated tetracycle **21**.

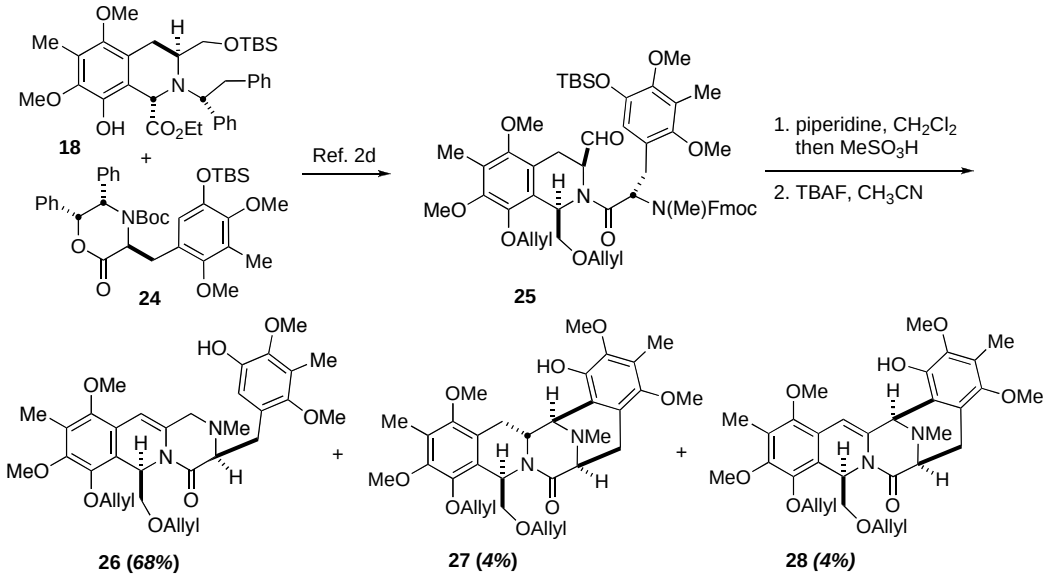
We have rationalized these results as follows: after formation of the iminium ion species **22**, rapid tautomerization led to the formation of **20** that presents a thermodynamically favored benzylic alkene. Spontaneous oxidation of this allylic tertiary amine subsequently occurs and generates the C_3-C_4 unsaturated iminium intermediate **23** that could then be engaged in the [1,3]-dipolar cycloaddition through the incipient azomethine ylide to form the observed C_3-C_4 unsaturated tetracycle **21** (Scheme 6).[14] Zhu recently observed a similar spontaneous air oxidation of an allylic secondary amine to a C_3-C_4 unsaturated species in connection with synthetic studies toward lemonomycin.[12c]



Scheme 6. Tautomerization/oxidation.

The fortuitous observation of the propensity of such allylic amines to suffer rapid and facile allylic oxidation encouraged us to apply this type of transformation to the pentacyclic framework of cribrostatin 4. The starting point for the synthesis of the allylic amine **26** was the amino aldehyde **25** obtained from tetrahydroisoquinoline **18** and amino acid precursor **24** as reported in our syntheses of 3-*epi*-renieramycin G and 3-*epi*-jorumycin (Scheme 7).[4d] The *N*-Fmoc protecting group in **25** was removed with piperidine

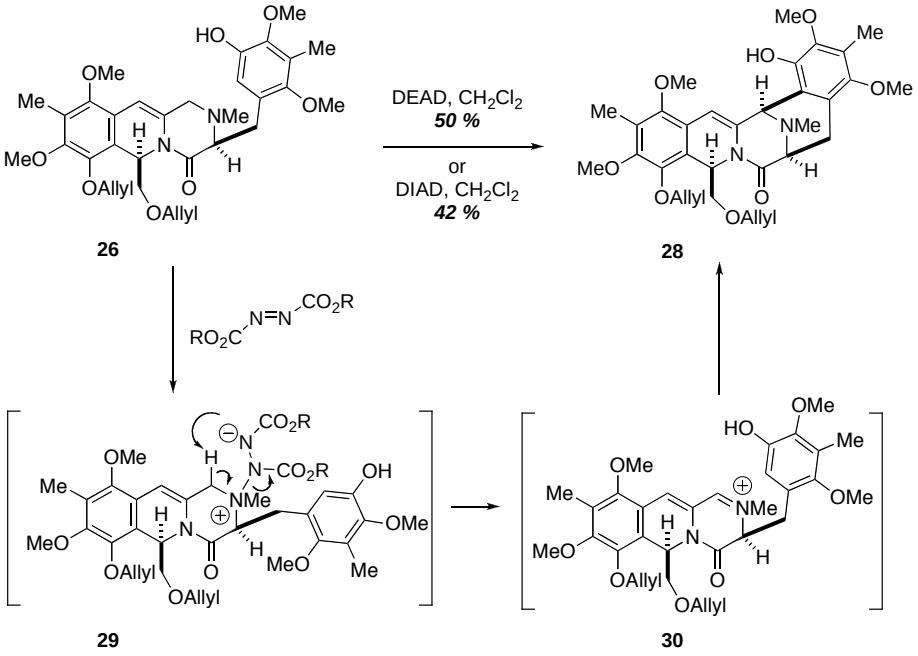
and methanesulfonic acid was subsequently added to promote the formation of the iminium species followed by migration of the double bond to the C_3-C_4 benzylic position. The *O*-TBS protecting group of the eastern phenol was then removed with TBAF to yield 68% of the desired allylic amine **26**, 4% of the *3*-epi-pentacycle **27**[2a] and significantly, we isolated 4% of the unsaturated pentacyclic compound **28** (Scheme 7). Moreover, after standing for ten days in an NMR tube in $CDCl_3$, a 10% conversion of **26** into **28** was also observed.



Scheme 7. Synthesis of allylic amine **26**.

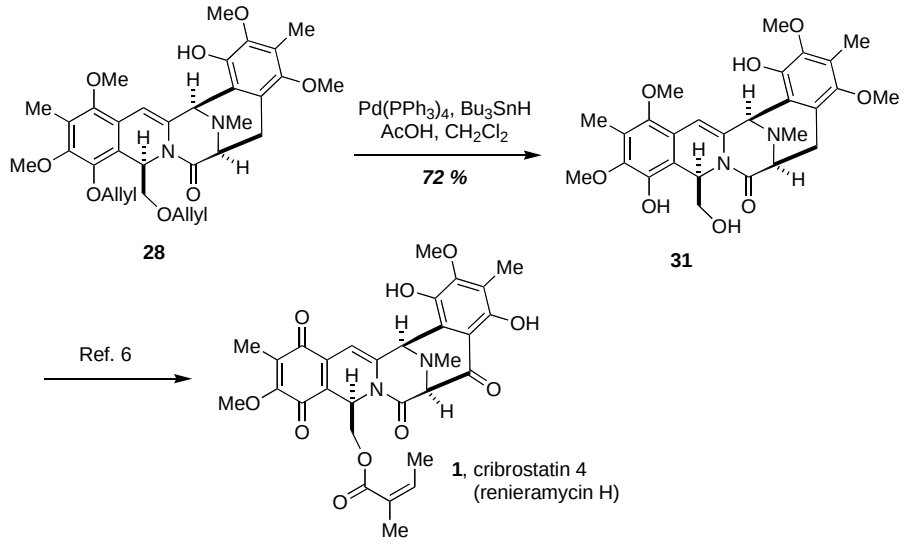
Encouraged by these promising results, we turned our efforts to find more efficient conditions for this oxidation. Numerous methods are described in the literature to form iminium species from tertiary amines.[14] Among them are the Polonovski-Potier reaction,[15] the use of NBS,[4a-c] $K_3Fe(CN)_6$[16] or TPAP-NMO.[17] These methods were all examined but unfortunately each gave mixtures of unidentified products with no attendant formation of pentacycle **28**. As noted above, TEMPO was successful in promoting the formation of the unsaturated skeleton of lemonomycin from **15** but here again in this case, only the starting amine **26** was recovered when treated with TEMPO. The literature contains a few reports concerning the oxidation of tertiary amines to iminium ion intermediates mediated by azodicarboxylates, such as DEAD (diethyl azodicarboxylate).[18] Indeed, treatment of **26** with DEAD in CH_2Cl_2 at room temperature for 15 h afforded the pentacycle **28** in 50% yield (Scheme 8). Replacing DEAD by DIAD (di-*iso*propyl azodicarboxylate) also afforded **28** in 42% yield. The mechanism for this oxidation presumably process through nucleophilic attack of the tertiary amine on the diazo bond of the azodicarboxylate species to form the zwitterionic intermediate **29**; subsequent β–H elimination generates

the key iminium ion species **30** immediately followed by cyclization to pentacyclic substance **28** (Scheme 8).



Scheme 8. DIAD-mediated oxidation of allylic amine **26**.

The allyl protecting groups of the phenol and the primary alcohol in **28** were then removed with $Pd(PPh_3)_4$ in the presence of *tri-n*-butyltin hydride and acetic acid to afford **31** (Scheme 9). As compound **31** constitutes an advanced intermediate utilized successfully in our recent asymmetric total synthesis of cribrostatin 4, this work thus represents a formal total synthesis of cribrostatin 4.[5]



Scheme 9. Formal total synthesis of cribrostatin 4.

In summary, synthetic studies on lemonomycin have led to the discovery of a very facile autoxidation of an electron-rich tricyclic dihydroisoquinoline. The incipient iminium ion was converted into an azomethine ylide that underwent 1,3 dipolar cycloaddition with *t*-butyl acrylate. We have thus found that azodicarboxylates can oxidize related tricyclic dihydroisoquinolines to generate synthetically useful amounts of a reactive, incipient iminium ion species that can be trapped in a Pictet-Spengler reaction to furnish the pentacyclic framework of cribrostatin 4. Currently, we are examining the reduction of the C_{21} amide moiety within this series into carbinolamine analogues (and functional equivalents) of cribrostatin 4, that is anticipated to lead to significantly more potent cytoxic agents capable of alkylating DNA.

EXPERIMENTAL

General procedures: Unless otherwise noted, materials were obtained from commercially available sources and used without further purification. THF and dichloromethane were degassed with argon and passed through a solvent system (J.C. Meyer of glass Contour). All reactions were conducted with flame- or oven-dried glassware under an inert atmosphere of argon unless otherwise noted. Chromatographic separations were performed with EM Science TLC plates (silica gel 60, F254, 20 x 20cm x 250μm) or with EM Science 230-240 mesh silica gel under positive air pressure. Infrared spectra were recorded on a Perkin-Elmer 1600 series FT-IR as thin films from methylene chloride and are reported as λ_{max} in wavenumbers (cm^{-1}). Nuclear magnetic resonance (NMR) spectra were acquired using Varian 300 or 400 spectrometers. NMR chemical shifts are given in parts per million (ppm) relative to internal $CHCl_3$, or DMSO. Mass spectra were obtained on Fisons VG Autospec. Optical rotations were obtained on a Rudolph Research automatic polarimeter Autopol III.

Compound 19: To a solution of **18** (6.65 g, 10.70 mmol) in CH_2Cl_2 was added Et_3N (4.5 mL, 32.10 mmol) dropwise at 0 ºC followed by AcCl (0.84 mL, 11.8 mmol). After 30 min at 0 ºC, the reaction mixture was filtered through silica gel. The filtrate was concentrated under reduced pressure and the crude acetate was used in the next step. A pressure tube containing the crude acetate (7.0 g) in absolute ethanol (280 mL) was purged with argon for 15 min, $Pd(OH)_2$ (700 mg) added to the mixture and the tube pressurized to 80 psi with hydrogen gas. The tube was then purged another 2 times with hydrogen. After 2 days, the pressure was released and the reaction filtered through a pad of celite. After concentration under reduced pressure, the residue was purified by chromatography (SiO_2, 1:1 hexane:AcOEt) affording the free amine. (1.2 g, 93% yield). $[\alpha]_D^{25}$= -20.6 (c 1.0, CH_2Cl_2). ^{1}H NMR (300MHz, $CDCl_3$): δ -0.08 (d, *J* = 4.5 Hz, 6H), 0.91 (s, 9H), 1.26 (t, *J* = 7.2 Hz, 3H), 2.22 (s, 3H), 2.27 (s, 3H), 2.38 (m, 1H), 2.76 (dd, *J* = 3.9, 16.5 Hz,1 H), 3.20 (m, 1H), 3.58 (m, 1H), 3.73 (s, 6H), 3.80 (dd, *J* = 9.9, 3.3 Hz, 1H), 4.18 (q, *J*=

14.4, 7.2 Hz, 2H), 4.69 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ -5.0, -4.9, 10.1, 14.62, 18.6, 20.8, 25.6, 26.2, 50.5, 55.8, 60.0, 61.0, 61.5, 67.0, 124.2, 124.8, 124.9, 138.1, 148.8, 154.7, 168.4, 172.1; IR (thin film): 2932, 2856, 1770, 1736, 1199, 1109, 837 cm^{-1}. HRMS (FAB+) calcd. for $C_{24}H_{40}NO_7Si$ (MH^+, m/z) 482.2574; found 482.2573**.**

To a solution of the free amine (2.0 g, 4.15 mmol) in CH_2Cl_2 (2 mL) was added EDCI (1.2 g, 6.23 mmol) followed by *N*-Bn, *N*-Boc glycine (2.2 g, 8.3 mmol). After stirring for two and half days, the reaction mixture was diluted with EtOAc (200 mL), washed with water (50 mL) brine (50 mL) and dried (Na_2SO_4). After concentration under reduced pressure, the resulting residue was purified by flash chromatography (SiO_2, 6:1 hexane:AcOEt) affording **19** (2.9 g, 97% yield). $[\alpha]_D^{25}$= +43.7 (c 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO, 120°C): δ -0.20 (d, *J* = 9.8 Hz, 6H), 0.78 (s, 9H), 1.12 (t, *J*= 7 Hz, 3H), 1.41 (s, 9H), 2.18 (s, 3H), 2.32 (s, 3H), 2.91- 3.09 (m, 2H), 3.27 (d, *J* = 15.8 Hz, 2H), 3.69 (s, 6H), 3.85- 4.11 (m, 3H), 4.21-4.35 (m, 2H), 4.35 (d, *J*=15.6 Hz, 1H), 4.47 (d, *J* = 15.6 Hz, 1H), 5.47 (s, 1H), 7.16- 7.41 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$, mixture of rotamers): δ -5.5, -5.3, 10.3, 14.1, 18.5, 21.1, 23.7, 26.1, 28.6, 28.7, 47.9, 51.0, 51.6, 60.1, 61.0, 62.0, 63.3, 63.6, 80.6, 121.9, 123.2, 126.3, 127.5, 127.8, 128.4, 128.7, 137.7, 138.1, 138.3, 150.0, 154.9, 156.1, 168.2, 169.8, 170.1. IR (thin film): 2931, 2857, 1780, 1743, 1703, 1667, 1411, 1120, 1117 cm^{-1}. HRMS (FAB+) calcd. for $C_{38}H_{57}N_2O_{10}Si$ (MH^+, m/z) 729.3783; found 729.3787.

Compound 15: To a solution of **19** (1.08 g, 1.5 mmol) in THF/ MeOH (16 mL/ 1 mL) was added TBAF (1M in THF, 1.8 mL, 1.8 mmol) and the reaction was stirred at rt for 18 h. After this time the reaction was quenched with sat. aq. NH_4Cl (5 mL) and diluted with EtOAc (200 mL). The organic layer was washed with brine and dried (Na_2SO_4). After concentration under reduced pressure the resulting residue was purified by flash chromatography (SiO_2, 3:1 hexane:AcOEt) affording the desired alcohol (0.82 g, 89% yield). $[\alpha]_D^{25}$= +68.8 (c 1.0, CH_2Cl_2). ^{1}H NMR (400MHz, DMSO, 120°C): δ 1.13 (t, *J* = 6.7 Hz, 3H), 1.42 (s, 9H), 2.19 (s, 3H), 2.33 (s, 3H), 2.93 (dd, *J* = 15, 5.4 Hz, 1H), 3.10 (bs, 1H), 3.25 (d, *J* = 15.7 Hz, 1H), 3.69 (s, 3H), 3.70 (s, 3H), 3.86–4.10 (m, 2H), 4.14–4.30 (m, 3H), 4.40–4.54 (m, 3H), 5.50 (s, 1H), 7.23–7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$, mixture of rotamers): δ 10.3, 14.1, 21.1, 23.9, 24.5, 28.6, 28.7, 48.0, 51.1, 52.7, 53.4, 53.5, 60.9, 61.3, 62.2, 63.8, 65.0, 80.9, 121.7, 123.3, 126.4, 127.5, 128.4, 128.7, 137.9, 138.1, 150.2, 154.5, 156.4, 168.1, 170.0. IR (thin film): 3463, 2937, 1778, 1699, 1411, 1200 cm^{-1}. HRMS (FAB+) calcd. for $C_{32}H_{43}N_2O_{10}$ (MH^+, m/z) 615.2918; found 615.2912.

To a solution of $(COCl)_2$ (0.17mL, 1.95 mmol) in CH_2Cl_2 (3.2 mL) at -78°C, was added DMSO (0.18 mL, 2.60 mmol) dropwise. After 5 min a solution of the preceding alcohol (400mg, 0.65 mmol) in CH_2Cl_2 (5 mL) was added dropwise. After a further 45 min, Et_3N (0.90 mL, 6.5 mmol) was added to the reaction mixture and it was kept at -78°C for 5 min and then warmed to 0°C. The reaction was then quenched with

sat. aq. NH_4Cl and diluted with CH_2Cl_2 (20 mL). The organic layer was washed with sat. aq. NH_4Cl (3 mL) followed by brine and dried (Na_2SO_4). After concentration under reduced pressure the resulting residue was purified by flash chromatography (SiO_2, 3:1 hexane:AcOEt) affording **15** (320 mg, 80% yield). $[\alpha]_D^{25}$= +50.8 (c 1.0, CH_2Cl_2).^{1}H NMR (400 MHz, DMSO, 120°C): δ 1.13 (t, *J* = 7.0 Hz, 3H), 1.41 (s, 9H), 2.17 (s, 3H), 2.33 (s, 3H), 3.11 (bs, 1H), 3.54 (m, 1H), 3.69 (s, 3H), 3.70 (s, 3H), 3.81–4.02 (m, 2H), 4.02–4.12 (m, 1H), 4.20 (d, *J* = 17.0, 1H), 4.37 (d, *J* = 15.6 Hz, 1H), 4.45 (d, *J* = 15.6 Hz, 1H), 4.97 (br s, 1H), 5.71 (s, 1H), 7.18–7.38 (m, 5H), 9.27 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers): δ 10.1, 13.9, 14.4, 20.9, 21.0, 22.0, 23.3, 28.5, 29.9, 47.1, 47.8, 50.9, 53.1, 53.4, 53.7, 60.0, 60.3, 60.8, 60.9, 61.1, 62.3, 62.8, 80.9, 81.1, 121.4, 121.8, 122.3, 126.8, 127.7, 128.1, 128.2, 128.6, 128.8, 137.5, 137.9, 150.3, 150.7, 153.9, 155.8, 168.2, 170.0, 173.1, 198.9, 199.3, 201.0. IR (thin film): 3450, 2978, 1776, 1741, 1411, 1200 cm^{-1}. HRMS (FAB+) calcd. for $C_{32}H_{41}N_2O_{10}$ (MH^+, m/z) 613.2761; found 613.2765.

Compounds 20 and 21: To a solution of **15** (165 mg, 0.27 mmol) in $CHCl_3$ (2.7 mL) was added TFA (1.10 mL, 13.50 mmol). The flask was left open to air and then sealed with a glass stopper. After stirring for 3.5 h, the solvent and TFA were removed under reduced pressure and the resulting residue was dissolved with $CHCl_3$ (2.7 mL). To this reaction mixture was added *t*-butyl acrylate (0.80 mL, 5.40 mmol) and Et_3N (0.37 mL, 2.7 mmol) at 0 ºC. The reaction was warmed to rt and stirred for 3 h. The reaction mixture was then diluted with EtOAc (20 mL) and washed with saturated NH_4Cl. The organic layer was washed with brine and dried (Na_2SO_4). After concentration under reduced pressure, the resulting residue was purified by flash chromatography (silica, 3:1 hexane:EtOAc) affording **21** (110 mg, 67% yield) and **20** (15 mg, 11%).

21: $[\alpha]_D^{25}$= -48.2 (c 1.0, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, *J* = 7.3 Hz, 3H), 1.44 (s, 9H), 2.13 (dd, *J* = 9.7, 13.9 Hz, 1H), 2.22 (s, 3H), 2.40 (s, 3H), 2.67 (m, 1H), 2.79 (dd, *J* = 4.6, 9.5 Hz, 1H), 3.71 (s, 3H), 3.72 (s, 3H), 3.82 (dd, *J* = 7.4 Hz, 1H), 3.93–4.31 (m, 4H), 4.17 (s, 1H), 5.80 (s, 1H), 6.27 (s, 1H), 7.20–7.43 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.9, 14.2, 21.1, 28.2, 32.1, 50.1, 51.3, 51.6, 60.9, 61.7, 62.3, 63.0, 64.1, 81.5, 97.8, 116.9, 120.9, 126.7, 127.3, 128.4, 128.9, 137.4, 138.2, 138.5, 150.3, 151.2, 168.2, 168.6, 170.9, 171.9. IR (thin film): 2978, 1741, 1692, 1202 cm^{-1}. HRMS (FAB+) calcd. for $C_{34}H_{41}N_2O_9$ (MH^+, m/z): 621.2812; found: 621.2805.

20: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (t, *J* = 7.3 Hz, 3H), 2.19 (s, 3H), 2.35 (s, 3H), 3.38-3.61 (m, 4H), 3.65 (s, 2H), 3.68 (s, 3H), 3.69 (s, 3H), 3.95 (m, 1H), 4.15 (m, 1H), 5.81 (s, 1H), 6.34 (s, 1H), 7.24–7.34 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.9, 14.1, 21.1, 50.8, 53.5, 57.5, 60.7, 60.9, 61.5, 62.4, 100.0,

117.6, 120.6, 126.6, 127.9, 128.7, 129.3, 133.1, 136.8, 138.1, 150.3, 151.4, 166.4, 168.1, 168.5. HRMS (FAB+) calcd. for $C_{27}H_{31}N_2O_7$ (MH^+, m/z): 495.2131; found: 495.2119.

Synthesis of **21** via NBS oxidation of **20** followed by 1,3 cycloaddition: To a solution of **20** (110 mg, 0.22 mmol) in $CHCl_3$ (3 mL) was added NBS (43.5 mg, 0.25 mmol) and the solution was heated to reflux for 45 min. The solution was cooled to 0°C and *t*-butyl acrylate (660 μL)was added followed by the dropwise addition of a solution of Et_3N (245 μL, 1.76 mmol) in CH_2Cl_2 (850 μL). The reaction mixture was stirred for 3 h at rt. The solvent was then removed under reduced pressure. The crude material was diluted with CH_2Cl_2 (5 mL) and then washed with sat. aq. $NaHCO_3$ (5 mL). The organic layer was dried with Na_2SO_4. After concentration the residue was purified by chromatography to afford **21** (48.2 mg, 35%).

Compound 26: To a solution of **25** (55 mg, 0.0580 mmol) in CH_2Cl_2 (4 mL) under argon was added piperidine (57 μL, 0.0580 mmol) and the reaction was stirred for 15 h at rt. Methansulfonic acid (150 μL) was subsequently added and the reaction was stirred at rt for an additional 2 h. The reaction mixture was then quenched with sat. aq. $NaHCO_3$ (5 mL) and diluted with CH_2Cl_2 (5 mL). The aqueous layer was washed with CH_2Cl_2 (2 x 5 mL) and the combined organic phases were dried (Na_2SO_4) and concentrated. The crude mixture obtained was then diluted in CH_3CN (4 mL) and TBAF (30 mg, 0.115 mmol) was added. The reaction mixture was stirred for 2 h at rt and then quenched with sat. aq. NH_4Cl (5 mL) and diluted with EtOAc (5 mL). The aqueous layer was extracted with EtOAc (2 x 5 mL) and the combined organic phases were dried (Na_2SO_4). After concentration under reduced pressure the resulting residue was purified by flash chromatography (SiO_2, 1:1 hexane:EtOAc) affording 2.7 mg of a 1:1 mixture of **27** (4%) and **28** (4%) along with the pure desired allylic amine **26** (23.5 mg, 68% yield) as a yellow oil. $[\alpha]_D^{25}$= + 118.4 (c 0.25, CH_2Cl_2). ^{1}HNMR ($CDCl_3$, 300 MHz): 2.17 (s, 3H), 2.23 (s, 3H), 2.36 (s, 3H), 3.24-3.04 (m, 2H), 3.38 (dd, *J* = 11.1, 3.9 Hz, 1H), 3.66-3.56 (m, 2H), 3.69 (s, 3H), 3.71 (s, 3H), 3.76-3.72 (m, 1H), 3.77 (s, 3H), 3.79 (s, 3H), 3.98-3.90 (m, 2H), 4.21 (dd, J = 12.6, 5.4 Hz, 1H), 4.58-4.53 (m, 2H), 5.14 (dd, *J* = 10.5, 1.8 Hz, 1H), 5.29-5.22 (m, 2H), 5.43 (dd, *J* = 16.8, 1.8 Hz, 1H), 5.59 (br s, 1H), 5.87 (s, 1H), 5.94-5.81 (m, 1H), 6.13 (dddd, *J* = 16.5, 10.8, 5.8, 5.4 Hz; 1H), 6.38 (dd, *J* = 8.7, 3.8 Hz, 1H), 6.81 (s, 1H). ^{13}CNMR ($CDCl_3$, 75 MHz): 9.4, 10.0, 31.5, 42.1, 46.4, 51.0, 60.4, 60.8, 60.9, 61.4, 66.7, 69.9, 71.7, 74.2, 101.2, 113.8, 117.2, 117.9, 120.3, 121.3, 124.4, 125.2, 128.2, 130.9, 134.2, 134.8, 144.4, 144.7, 145.2, 149.3, 150.6, 168.8. TLC: Rf = 0.65 (SiO_2, 1:3 hexane:EtOAc). IR (thin film): 3386, 2936, 2854, 1673, 1634, 1462, 1415, 1239, 1109, 1061, 1007. HRMS (FAB+) calcd for $C_{33}H_{43}N_2O_8$ (MH^+, m/z) 595.3019; found 595.3017.

Compound 28: To a solution of **26** (17.0 mg, 0.029 mmol) in CH_2Cl_2 (4 mL) was added a solution of DEAD (40% in toluene) (26 mg, 0.06 mmol) in CH_2Cl_2 (0.3 mL) over 15 min and the reaction was stirred for 5 h at rt. TLC showed that the reaction was not complete and again, a solution of DEAD (40% in toluene) (25 mg, 0.06 mmol) in CH_2Cl_2 (0.3 mL) was added over 15 min and the reaction was stirred for 15 h at rt The reaction mixture was then quenched with sat. aq. $NaHCO_3$ (4 mL) and diluted with CH_2Cl_2 (2 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 4 mL) and the combined organic phases were dried (Na_2SO_4). After concentration under reduced pressure the resulting residue was purified by Preparative TLC (SiO_2, 3:7 hexane:AcOEt) affording the desired unsaturated pentacycle **28** (8.5 mg, 50%) as a yellow film. $[\alpha]_D^{25}$= + 3.5 (c 0.28, CH_2Cl_2).^{1}HNMR ($CDCl_3$, 300 MHz): 2.17 (s, 3H), 2.19 (s, 3H), 2.54 (s, 3H), 3.10-2.98 (m, 2H), 3.20-3.12 (m, 2H), 3.41-3.25 (m, 2H), 3.65 (s, 3H), 3.72-3.69 (m, 1H), 3.73 (s, 3H), 3.75 (s, 3H), 3.76 (s, 3H), 4.51 (app d, *J* = 5.8 Hz, 2H), 4.63 (s, 1H), 4.91-4.83 (m, 2H), 5.41-5.21 (m, 3H), 5.55 (s, 1H), 6.09 (dddd, J = 17.1, 10.5, 5.8, 5.8 Hz, 1H), 6.19 (dd, J = 7.5, 3.9 Hz, 1H), 6.26 (s, 1H). ^{13}CNMR ($CDCl_3$, 75 MHz): 9.4, 9.7, 28.9, 41.7, 46.9, 56.4, 60.2, 60.4, 60.8, 61.0, 61.4, 70.1, 71.9, 74.2, 102.5, 116.4, 117.9, 119.7, 120.3, 121.4, 122.2, 122.9, 125.1, 132.4, 134.2, 135.1, 142.2, 143.8, 144.7, 149.6, 149.9, 150.7, 168.2. TLC: Rf = 0.35 (SiO_2, 1:3 hexane:EtOAc). IR (thin film): 3306, 2930, 2851, 1675, 1637, 1463, 1414, 1108, 1058, 1005 cm^{-1}. HRMS (FAB+) calcd for $C_{33}H_{41}N_2O_8$ (MH^+, m/z) 593.2863; found 593.2847.

Compound 31: To a solution of **28** (5.0 mg, 0.00840 mmol) in CH_2Cl_2 (1 mL) was successively added AcOH (7.5 mg, 0.126 mmol), $Pd(PPh_3)$ (5 mg, 0.00433 mmol), and Bu_3SnH dropwise (17.7 μL, 0.0672 mmol). The reaction was stirred 2 h, then quenched with sat. aq. $NaHCO_3$ (3 mL) and diluted with CH_2Cl_2 (3 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 3 mL) and the combined organic phases were dried (Na_2SO_4). After concentration under reduced pressure the resulting residue was purified by Preparative TLC (SiO_2, AcOEt) affording the desired triol **31** (3.1 mg, 72%) as a white film. $[\alpha]_D^{25}$= +5.3 (c 0.15, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): 1.80 (br, 1H), 2.19 (s, 6H), 2.54 (s, 3H), 3.20-3.15 (m, 3H), 3.31 (dd, *J* = 10.3, 5.1 Hz, 1H), 3.62 (s, 3H), 3.70 (s, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.73-3.70 (m, 1H), 4.65 (s, 1H), 5.61 (s, 1H), 5.75 (br, 1H), 6.10 (dd, *J* = 6.9, 5.1 Hz, 1H), 6.27 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 9.9, 29.4, 41.7, 49.3, 56.5, 60.4, 60.8, 61.0, 61.1, 61.7, 64.5, 102.9, 114.3, 119.4, 119.9, 121.7, 123.4, 124.0, 132.1, 142.0, 142.3, 144.1, 147.2, 149.9, 169.3. IR (thin film): 3345, 2930, 2855, 1633, 1465, 1414, 1308, 1257, 1107, 1054, 1003, 798, 736. HRMS (FAB+) calcd for $C_{27}H_{33}N_2O_8$ (M+H^+, m/z) 513.2236; found 513.2237. This substance proved to be identical to that obtained by a distinct route previously.[6]

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