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QUINONE-CYCLIZED PORFIROMYCINS

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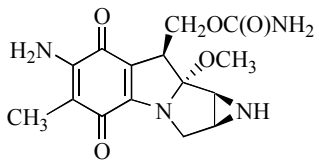
Abstract – Mitomycin C and porfiromycin cyclization has been implicated in the pathway for KW-2149 and BMS-181174 function. The importance of the porfiromycin C(7) structure and nucleophile for quinone cyclization was determined showing that both C(7) and C(8) annulation processes occur.

Mitomycin C (**1**) is extensively used in cancer chemotherapy[1] and serves as the prototypical bioreductive alkylating agent.[2] Since its clinical introduction over 600 semi-synthetic mitomycin analogues have been prepared in an effort to identify more efficacious agents.[3] In recent years, two mitomycins, KW-2149[4,5] (**2**) and BMS-181174[6,7] (**3**), have attracted extensive attention. Both compounds have undergone clinical trials, and **2** advanced to Phase II testing.[8]



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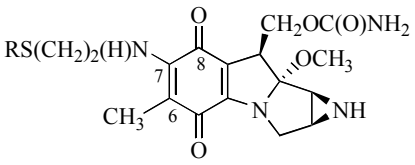
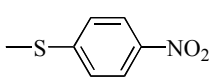


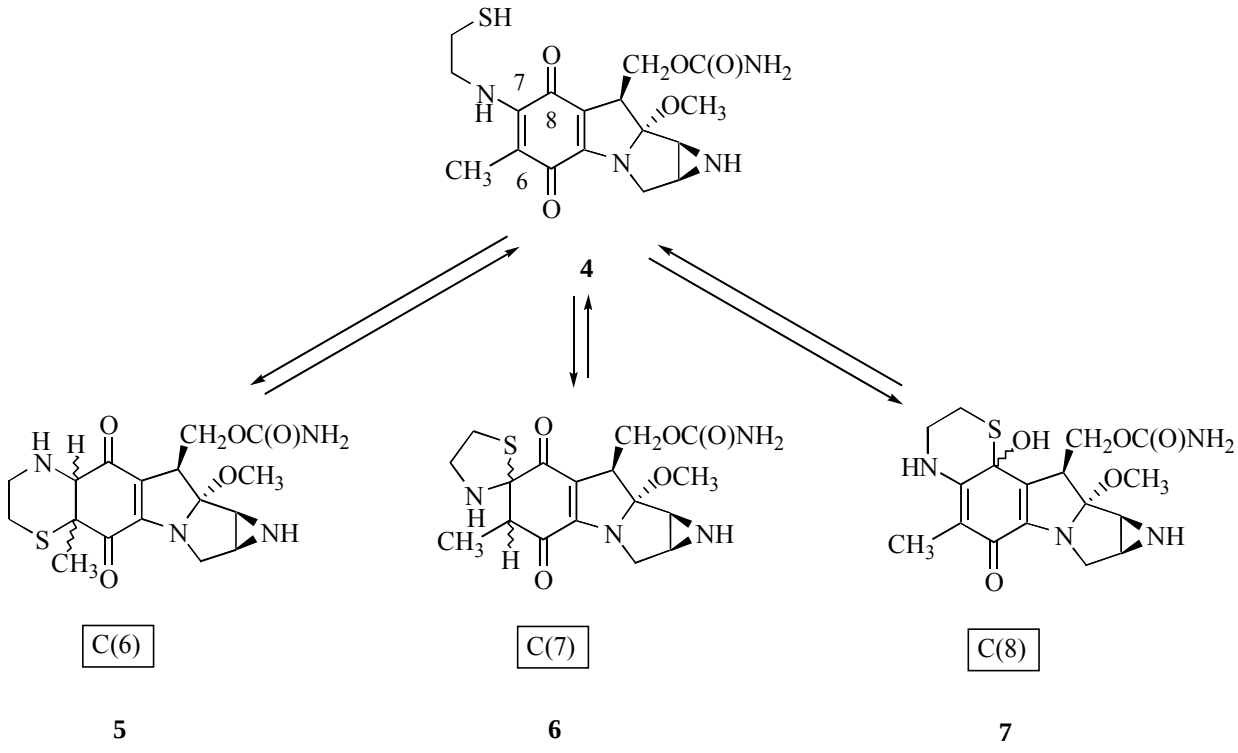
2 KW-2149 R = $-S(CH_2)_2N(H)C(O)(CH_2)_2CH(NH_2)CO_2H$

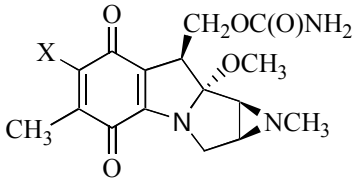
3 BMS-181174 R = 

4 R = H

Speculation exists as to the function of **2** and **3**. Structurally, they have much in common with **1**. Only the C(7) substituent in **1-3** differ; in **1**, the C(7) moiety is an amino unit while in **2** and **3** it is an aminoethylene disulfide group. The mechanisms proposed for **2** and **3** function are different than **1**.[5f,k,9-11] These pathways suggest that external thiol-mediated (*e.g.*, glutathione, serum albumin) disulfide cleavage of the C(7) aminoethylene disulfide unit is instrumental for drug function. In several reports, 7-*N*-(2'-mercaptoethyl)mitomycin C (**4**) has been proposed as the key intermediate, and suggestions have been made that this thiol undergoes quinone cyclization at the C(6), C(7) or C(8) sites to generate the intermediate cyclized adducts (**5-7**) (Figure 1).[9-11] In 1999, Wang and Kohn reported HPLC profiles for reactions designed to generate **4** and the corresponding porfiromycin thiol (**8**).[11] Porfiromycin (**9**) is an analogue of mitomycin C (**1**) in which the N(1a) hydrogen has been replaced with a methyl moiety.[2] The chromatograms for these reactions showed *multiple* peaks, which upon addition of thiol trapping reagents produced a single, major adduct corresponding to the modified thiol. These findings were consistent with the notion that thiols (**4**) and (**8**) cyclized at one or more sites to give an equilibrium isomeric mixture.[11] Attempts to characterize these adducts were unsuccessful.

Figure 1. Origin of Multiple HPLC Peaks





8 X = $N(H)CH_2CH_2SH$

9 X = NH_2

15 X = OCH_3

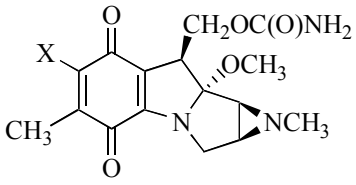
In this study, we report the synthesis and chemical properties of novel mitomycins designed to undergo quinone cyclization. We show that cyclization can proceed at the quinone C(7) and C(8) sites, and that cyclization depends upon the structure of the C(7) substituent.

RESULTS AND DISCUSSION

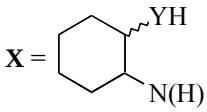
A. Selection of Compounds

In this study we asked whether nucleophiles tethered at the C(7) porfiromycin site cyclize to give C(6), C(7) and C(8) adducts (Figure 1). We prepared two porfiromycin series (Figure 2: **A** and **B**).

Figure 2. Substituted Porfiromycins

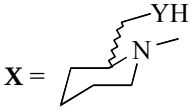


Series **A**



Y = O, NH, S

Series **B**

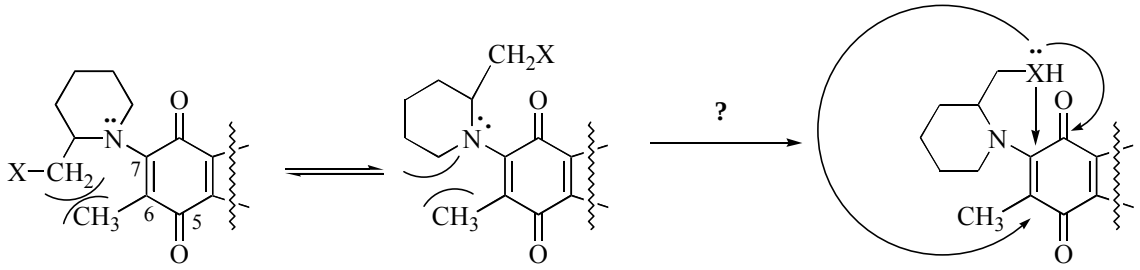


Y = O, S

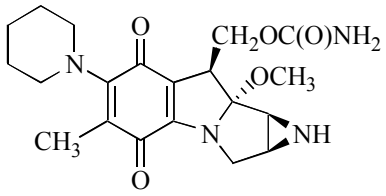
Porfiromycin analogues, rather than mitomycin derivatives, were synthesized to prevent N(1a)-C(5a) cyclization processes.[12] Series **A** contained a C(7)-substituted cyclohexylamino unit, and series **B** had a C(7)-substituted piperidino moiety. In both series, we maintained a two-carbon (C2) bridge between the C(7) amino unit and the terminal nucleophile. We retained this structural factor since a similar spatial relationship existed in **2** and **3**.[13] Structural analogues of **2** and **3** with linkers longer than C2 were less cytotoxic than **2** and **3**.[13] Furthermore, we expected that a C2 bridge would entropically promote ring cyclization. The C(7)-piperidino unit in **B** (Figure 2) distinguished this set of compounds from the corresponding C(7)-amino units in series **A** compounds and **1**-**3**. Structural models predicted that C(7)-piperidino steric interactions with the C(6)-methyl moiety would result in the loss of resonance

interactions of the C(7)-piperidino nitrogen with the C(7)-C(6)-C(5)-O(5) conjugated system and decrease the electron density within the quinone system (Figure 3).

Figure 3. Potential Steric Interactions in C(7)-Piperidinoporfiromycins Promoting Quinone Cyclization



This notion is supported by the reported quinone reduction potentials for **1** ($E_{½}$ = -0.45 V) and 7-piperidinomitomycin[14] (**10**) ($E_{½}$ = -0.27 V),[15] indicating that **10** is more readily converted into the corresponding hydroquinone than is **1**. We also expected that the loss of planarity of the C(7) piperidino-substituent with the quinone system would facilitate cyclization processes (Figure 3).



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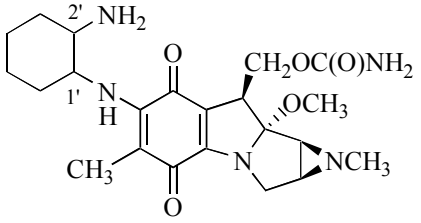
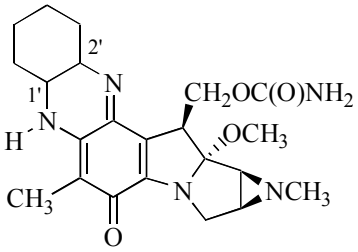
B. Series A Porfiromycins

The series **A** 2'-amino C(7)-cyclohexylaminoporiformycins (**11-14**) were the first compounds slated for synthesis. In 1989, Kyowa Hakko Kogyo scientists reported coupling a diastereomeric 1,2-diaminocyclohexane mixture with mitomycin F (**15**).[16] The product mixture was not separated but was assigned as C(8)-imino porfiromycins (**16-19**). We were anxious to learn if C(8) cyclization depended upon cyclohexane diamine geometry. Beginning with enantiomerically pure (1*S*, 2*S*)-*trans*- (**20**) and (1*R*, 2*R*)-*trans*-(**21**) 1,2-diaminocyclohexanes, condensation with **15** in MeOH gave C(8)-iminoporfiromycins (**16**) and (**17**), respectively. Similarly, treatment of **15** with *cis*-1,2-diaminocyclohexane (**22**) gave **18**[17] and **19**[17] as an 1:1 diastereomeric mixture (HPLC, TLC analyses). The spectral data for **16-19** were in agreement with the proposed structures, showing that imine formation was favored irrespective of cyclo-hexanediamine geometry. In particular, we observed for imines (**16-19**) a molecular ion peak in the low- and high-resolution MS. In the ^{13}C NMR for **16** and **17**, a single carbonyl peak (~179 ppm) was detected along with an additional downfield signal at ~153 ppm. The peak at ~153 ppm was diagnostic for the C(8) imine carbon.[10,18]





11 1'(*S*), 2'(*S*)

12 1'(*R*), 2'(*R*)

13 1'(*S*), 2'(*R*)

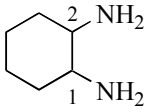
14 1'(*R*), 2'(*S*)

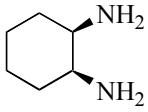
16 1'(*S*), 2'(*S*)

17 1'(*R*), 2'(*R*)

18 1'(*S*), 2'(*R*)

19 1'(*R*), 2'(*S*)





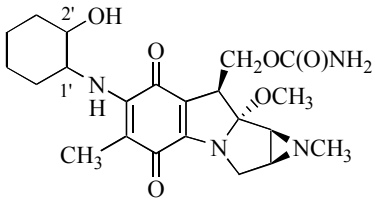
20 1(*S*), 2(*S*)

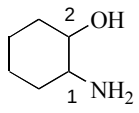
21 1(*R*), 2(*R*)

22

Our findings documented that the quinone C(8) site was prone to nucleophilic modification, a finding in agreement with earlier reports that mitomycins containing a C(7) acyclic terminal amino substituent underwent C(8) imine formation.[10,16,18]

The second set of series **A** compounds consisted of compounds (**23-26**). We prepared porfiromycins (**23**)[17] and (**24**)[17] by treating **15** with commercially available *trans*-2-aminocyclohexanol·HCl (**27**) and triethylamine in MeOH. Using the same procedure, we prepared **25**[17] and **26**[17] from **15** and *cis*-2-aminocyclohexanol·HCl (**28**).[19] The diastereomeric porfiromycins (**23-26**) were separated by PTLC. The low- and high-resolution MS spectra for **23-26** exhibited a molecular ion peak ($[M+1]^+$). The ^{13}C NMR spectra for **23-26** showed chemical shifts for the quinone ring system comparable to **9**,[20] documenting that the 2' hydroxyl unit did not cyclize at any of the quinone ring sites.





23 1'(*S*), 2'(*S*)

24 1'(*R*), 2'(*R*)

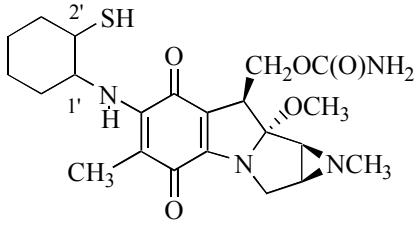
25 1'(*S*), 2'(*R*)

26 1'(*R*), 2'(*S*)

27 1(*S*),2(*S*) + 1(*R*),2(*R*)

28 1(*S*),2(*R*) + 1(*R*),2(*S*)

We then attempted to learn if (**29**) would undergo quinone cyclization. Replacement of the alcoholic residue in **23-26** with the nucleophilic thiol unit should foster cyclization. Unfortunately, attempts to prepare porfiromycins (**29**) using a diastereomeric mixture of *cis*- and *trans*-2-aminocyclohexanethiol·HI[21] (**30**) and **15** in basic MeOH were unsuccessful. Under deaerated conditions, we obtained only disulfides (**31**) and (**32**). High-resolution MS spectroscopy (CI) of the mixture showed signals at 592.262 27 ($[M+1]^+$) and 922.372 97 ($[M]^+$) corresponding to the molecular ion peaks of **31** and **32**, respectively. We verified this finding by preparing disulfide (**33**) from **30** and H_2O_2 and then treating the disulfide mixture with **15** in basic MeOH to give **31** and **32** (data not shown). The origin of **31** and **32** has not been determined. We suspect that **30** oxidatively dimerized to **33** under the reaction conditions and then **33** reacted with **15** to give **31** and **32**. A similar finding has been reported by Kono and coworkers for the reaction of mitomycin A with cysteamine.[13]



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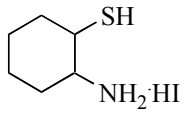

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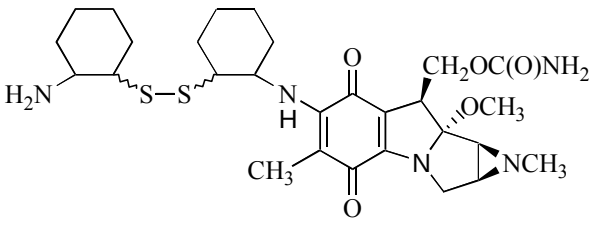

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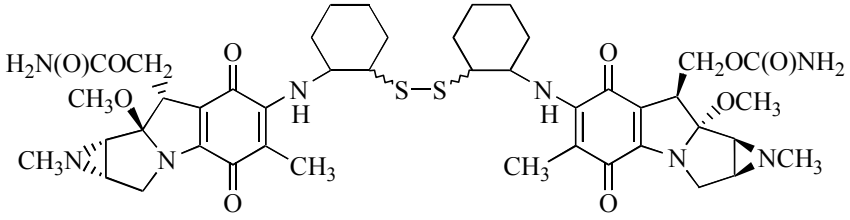

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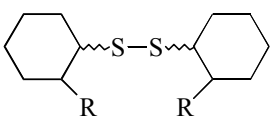
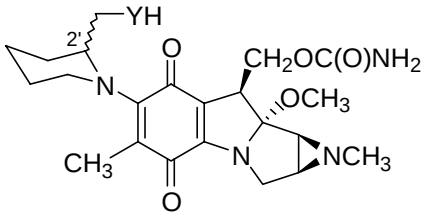
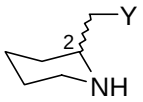


33 R = NH_2·HI

B. Series B Porfiromycins

Series **B** porfiromycins consisted of **34**-**37**. We envisioned that treatment of **15** with commercially available (*S,R*)-2-piperidinemethanol (**38**) would initially provide **34** and **35**.





34 Y = O, 2'(*S*)

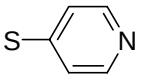
35 Y = O, 2'(*R*)

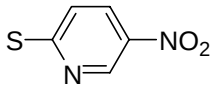
36 Y = S, 2'(*S*)

37 Y = S, 2'(*R*)

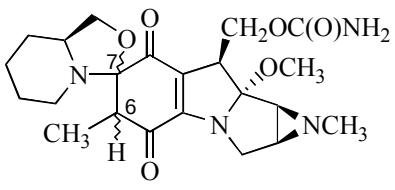
38 Y = OH, 2(*S*) + 2(*R*)

46 Y = SH, 2(*S*) + 2(*R*)

52 Y =  , 2(*S*) + 2(*R*)



When both compounds were mixed in MeOH, we observed the rapid disappearance of **15** and the appearance of two major orange products (HPLC and TLC analyses). PTLC separation of the mixture provided **39**[17] and **40**[17] (43% isolated yield). Spectroscopic studies documented that these compounds were C(7)-*N,O*-spiro adducts. We observed molecular ion peaks in the low- and high-resolution MS spectrum for **39** and **40**. In the ^{1}H NMR spectrum, the C(6) methyl protons appeared as an upfield doublet (~δ 1.31) and was coupled to the downfield C(6) methine quartet (~δ 3.34). Further structural support came from the ^{13}C NMR spectrum. First, the resonances for C(6) and C(7) appeared at ~52.0 ppm and ~97.8 ppm, upfield of the corresponding 105.1 and 147.3 ppm peaks for **9**. Second, the piperidino C(2') methylene carbon resonated ~10 ppm downfield of the corresponding signal in **38**. Finally, the quinone carbonyl carbon signals (191.7-193.4 ppm) appeared downfield (~15 ppm) of those in **9**.



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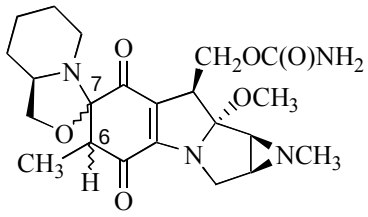
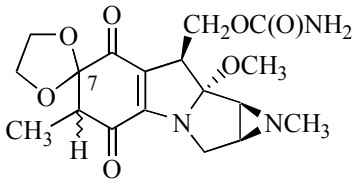


40

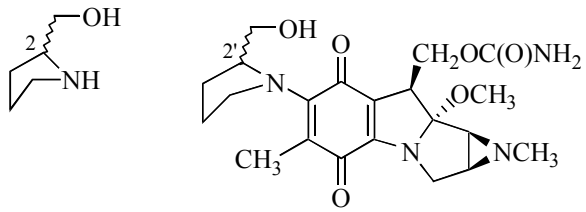
The observation of only two major products from the reaction of **38** with **15** (HPLC analysis) was surprising and indicated that C(7) spiro-cyclization proceeded with a high degree of stereospecificity. Cyclization introduced new stereocenters at C(6) and C(7). The isolation of **39** and **40** demonstrated that appropriate nucleophiles tethered at the porfiromycin C(7) position can react at the C(7) site to give

quinone cyclized adducts. A comparable product was reported by Kasai and coworkers, who prepared the C(7) cyclized **41** adduct in which both C(7) heteroatoms were oxygen.[22]



41

The generality of this C(7) spiro-cyclization reaction was briefly explored. Treatment of **15** with (*S*)-(**42**)- and (*R*)-(**43**)-pyrrolidinemethanol in MeOH led to the rapid production of **44** and **45**, respectively. The spectral properties for **44** and **45** documented C(7) pyrrolidinemethanol substitution and that C(7) modification proceeded *without* spiro-cyclization.

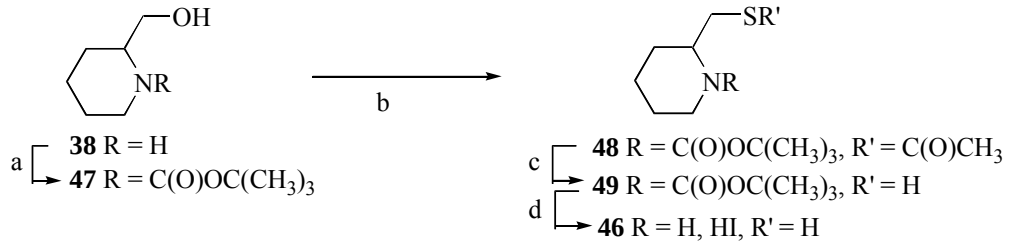


42 2(*S*) **44** 2'(*S*)

43 2(*R*) **45** 2'(*R*)

We next attempted to synthesize **36** and **37** using (*S,R*)-2-piperidinemethanethiol (**46**) and **15**. We prepared **46** as the hydroiodide salt from **38** using an improved procedure (Scheme 1). (*S,R*)-2-Piperidinemethanol (**38**) was protected as the (*N*)-*t*-Boc derivative (**47**)[23] and then treated with DEAD, PPh_3 and thiolacetic acid to give the Mitsunobu-coupled product (**48**). Hydrolysis of thioacetate moiety in **48** gave **49**; removal of the *t*-Boc group with trimethylsilyl iodide provided **46** in 56% overall yield from **38**.

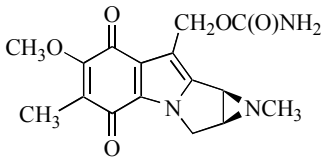
Scheme 1. Synthesis of 2-Piperidinemethanethiol Hydrogen Iodide (**46**)



a, *t*-Boc anhydride, THF, 85%; b, PPh_3, DEAD, $CH_3C(O)SH$, 71%;
c, K_2CO_3, CH_3OH-H_2O, 93%, d, TMSI, $CDCl_3$, 99%.

Addition of **46** to **15** in the presence of K_2CO_3 and triethylamine in a deaerated (Ar) methanolic solution led to the rapid formation of *N*(1a)-methyl-7-methoxyaziridinomitosene[24] (**50**) as the major product (HPLC and TLC analyses). Compound (**50**) has been prepared from **15** by a sequential reduction-

oxidation route.[24] We suspect a similar process occurred in this transformation. Previous reports have documented that thiols can reduce mitomycin A (**51**).[13] Two additional findings supported this notion. First, repetition of the reaction in *non*-deaerated methanolic solutions showed little reaction for extended time periods (2 d) (HPLC analysis). Second, treatment of **15** with the 4-pyridyl (**52**) and 5-nitro-2-pydridyl (**53**) disulfide analogues of **46** in non-deaerated basic MeOH solutions produced little product and the recovery of **15** (data not shown).[25]



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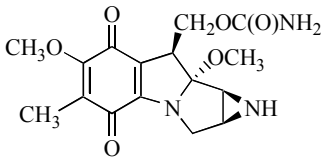

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CONCLUSIONS

Our findings document that nucleophiles appended to the quinone ring within the porfiromycin skeleton can generate C(7)- and C(8)-modified adducts. These results suggest that the multiple peaks observed upon generation of thiols (**4**) and (**8**) likely resulted from quinone modification. The pharmacological ramifications of these cyclization processes and whether these transformations contribute to drug efficacy are topics of current investigation.

EXPERIMENTAL

General Methods. UV-visible spectra were obtained using a Cary-3Bio UV-visible spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were taken on General Electric QE 300 MHz and Bruker AMX 600 MHz NMR instruments. Low-resolution and high-resolution (CI) MS spectral investigations were conducted at the University of Texas at Austin by Dr. M. Moini. The low-resolution MS studies were run on a Finnegan MAT-TSQ-70 instrument and the high-resolution MS studies were conducted on a Micromass ZAB-E spectrometer tuned to a resolution of 10,000 (10% valley definition). HPLC analyses were conducted with the following Waters Associate Units: instrument A: 510 A pump, 510 B pump, Model 680 gradient controller, Model 490 multiwavelength detector, U6K injector; instrument B: 515 A pump, 515 B pump, Millennium chromatography manager, Waters 996 photodiode array detector, Rheodyne 7725i manual injector. In both cases the column was fitted with a μBondapak guardpak pre-column. The product analyses were conducted with a C_{18} μBondapak (stainless steel) column (3.9 x 300 mm) using the following linear gradient condition: 90% A (aqueous 0.025 M triethylammonium acetate, pH 6.5), 10% B (acetonitrile) isocratic for 5 min, then from 90% A, 10% B to 45% A, 55% B in 30 min. The flow rate was 1 mL/min, and the eluent was monitored at 313 and 365 nm on instrument A, and from 200 to 400 nm on instrument B. The HPLC solvents were filtered (aqueous solution with Millipore HVLP, 0.45 mm;

acetonitrile with Millipore HV, 0.45 mm) and degassed before utilization. Thin layer chromatography was run on general purpose silica gel plates (20 x 20 cm; Aldrich No. Z12272-6). Tetrahydrofuran was distilled from Na metal and benzophenone. The Ar used was O_2-free (O_2 < 5 ppm).

General Method for the Synthesis of C(7)-Aminosubstituted Porfiromycins. To an anhydrous methanolic solution of **15** (1 equiv) was added a methanolic solution of the amine (0.5-10 equiv). The reaction solution was stirred at rt (1-2 d) and the solvent was removed *in vacuo* and where appropriate purified by PTLC (10% MeOH:$CHCl_3$).

By using this procedure following compounds were prepared.

Compound (16). Using **15** (5.0 mg, 14 μmol), **20** (1.6 mg, 15 μmol) and MeOH (1.3 mL) gave (5.9 mg, 100%) **16** (2 d) as a red solid: HPLC t_R: 24.6 min (instrument B); R_f 0.50 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 223, 365 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.10-1.14 (m, 1 H), 1.27-1.43 (m, 3 H), 1.52 (br dd, *J* = 12.0, 12.0 Hz, 1 H), 1.63 (br d, *J* = 12.0 Hz, 1 H), 1.95 (br d, *J* = 11.7 Hz, 1 H), 1.97 (s, 3 H), 2.12 (dd, *J* = 1.5, 4.5 Hz, 1 H), 2.25 (br d, *J* = 12.0 Hz, 1 H), 2.31 (s, 3 H), 2.61 (d, *J* = 4.5 Hz, 1 H), 2.90 (ddd, *J* = 3.6, 11.1, 11.7 Hz, 1 H), 3.05 (ddd, *J* = 3.6, 11.7, 12.0 Hz, 1 H), 3.28 (s, 3 H), 3.60 (dd, *J* = 1.5, 12.3 Hz, 1 H), 4.06 (dd, *J* = 4.5, 11.1 Hz, 1 H), 4.45 (d, *J* = 12.3 Hz, 1 H), 5.07 (dd, *J* = 10.5, 11.1 Hz, 1 H), 5.84 (dd, *J* = 4.5, 10.5 Hz, 1 H), 6.71 (s, 1 H); ^{13}C NMR (pyridine-d_5, 75 MHz) 8.8, 24.9, 26.2, 31.8, 33.6, 43.8, 44.1, 48.0, 48.6, 49.7, 51.7, 55.0, 63.2, 63.9, 106.8, 107.0, 115.9, 142.5, 148.3, 153.5, 158.9, 179.7 ppm; MS (+CI, methane) m/z 428 $[M+1]^+$; M_r (+CI, methane) 428.228 95 $(M+1)^+$ (calcd for $C_{22}H_{30}N_5O_4$, 428.229 78).

Compound (17). Using **15** (6.0 mg, 17 μmol), **21** (1.9 mg, 17 μmol) and MeOH (1.3 mL) gave (7.1 mg, 100%) **17** (1 d) as a red solid: HPLC t_R: 23.5 min (instrument B); R_f 0.47 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 223, 368 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.14-1.24 (m, 2 H), 1.28-1.35 (m, 2 H), 1.52 (br d, *J* = 11.4 Hz, 1 H), 1.63 (br d, *J* = 9.0 Hz, 1 H), 1.96 (br d, *J* = 11.4 Hz, 1 H), 2.00 (s, 3 H), 2.14 (dd, *J* = 1.5, 4.5 Hz, 1 H), 2.31 (s, 3 H), 2.31-2.35 (m, 1 H), 2.61 (d, *J* = 4.5 Hz, 1 H), 2.78 (ddd, *J* = 3.6, 11.4, 12.0 Hz, 1 H), 3.15 (ddd, *J* = 3.6, 11.7, 12.0 Hz, 1 H), 3.28 (s, 3 H), 3.58 (dd, *J* = 1.5, 12.6 Hz, 1 H), 4.13 (dd, *J* = 4.2, 11.1 Hz, 1 H), 4.37 (d, *J* = 12.6 Hz, 1 H), 5.08 (dd, *J* = 10.5, 11.1 Hz, 1 H), 5.45 (dd, *J* = 4.2, 10.5 Hz, 1 H), 6.88 (s, 1 H); ^{13}C NMR (pyridine-d_5, 75 MHz) 8.9, 24.9, 26.1, 31.6, 33.3, 43.8, 44.3, 46.3, 48.6, 49.7, 51.8, 54.8, 63.2, 63.8, 106.6, 107.2, 116.7, 142.5, 148.6, 153.5, 158.9, 179.9 ppm; MS (+CI, methane) m/z 428 $[M+1]^+$; M_r (+CI, methane) 428.228 81 $(M+1)^+$ (calcd for $C_{22}H_{30}N_5O_4$, 428.229 78).

Compounds (18)[17] and (19).[17] Using **15** (3.2 mg, 9.0 μmol), **22** (1 mg, 9.0 μmol) and MeOH (0.3 mL) gave **18** and **19** as a mixture (3.8 mg, 100%): HPLC t_R: 22.0 and 22.4 min (instrument B); R_f 0.43 and

0.46 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 223, 367 nm; MS (+CI, methane) m/z 428 $[M+1]^+$; M_r (+CI, methane) 428.230 03 $[M+1]^+$ (calcd for $C_{22}H_{30}N_5O_4$, 428.229 78).

7-*N*-(*trans*-2-Hydroxycyclohexyl)porfiromycins (23) and (24). Using **15** (9 mg, 25 μmol), **27** (8 mg, 51 μmol), TEA (7 μL, 51 μmol) and MeOH (2.5 mL) provided compounds (**23**) and (**24**) (1 d) as a solid.

Compound (23)[17] (3.7 mg, 32%): HPLC t_R: 23.7 min (instrument B); R_f 0.49 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 220, 369 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.22-1.39 (m, 4 H), 1.72-1.77 (m, 2 H), 1.94-1.98 (m, 1 H), 2.01 (s, 3 H), 2.02-2.07 (m, 1 H), 2.27 (s, 3 H), 2.28-2.34 (m, 2 H), 3.18 (s, 3 H), 3.45-3.48 (m, 1 H), 3.47 (d, J = 12.9 Hz, 1 H), 3.57 (dd, J = 4.5, 10.8 Hz, 1 H), 3.71-3.79 (m, 1 H), 4.25 (d, J = 12.9 Hz, 1 H), 4.35 (dd, J = 10.5, 10.8 Hz, 1 H), 4.70 (dd, J = 4.5, 10.5 Hz, 1 H), 6.22 (d, J = 10.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 10.3, 24.2, 24.5, 33.6, 33.7, 42.7, 43.1, 43.4, 46.4, 49.9, 50.0, 59.9, 62.7, 74.7, 104.3, 106.1, 109.9, 147.3, 155.4, 156.6, 176.2, 179.0 ppm; MS (+CI, methane) m/z 447 $[M+1]^+$; M_r (+CI, methane) 447.225 03 $(M+1)^+$ (calcd for $C_{22}H_{31}N_4O_6$, 447.224 36).

Compound (24)[17] (3.2 mg, 28%): HPLC t_R: 25.2 min (instrument B); R_f 0.40 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 220, 369 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25-1.36 (m, 4 H), 1.72-1.78 (m, 2 H), 1.94-1.98 (m, 1 H), 2.02 (s, 3 H), 2.04-2.09 (m, 1 H), 2.27 (s, 3 H), 2.29 (d, J = 4.2 Hz, 1 H), 2.34 (d, J = 4.2 Hz, 1 H), 3.18 (s, 3 H), 3.44-3.48 (m, 1 H), 3.47 (d, J = 12.9 Hz, 1 H), 3.57 (dd, J = 4.5, 10.5 Hz, 1 H), 3.72-3.79 (m, 1 H), 4.25 (d, J = 12.9 Hz, 1 H), 4.34 (dd, J = 10.5, 10.5 Hz, 1 H), 4.69 (dd, J = 4.5, 10.5 Hz, 1 H), 6.20 (d, J = 10.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 10.4, 24.2, 24.5, 33.5, 33.6, 42.8, 43.0, 43.4, 46.2, 49.9, 50.0, 59.8, 62.7, 74.7, 104.3, 106.2, 110.0, 147.3, 155.5, 156.6, 176.3, 179.1 ppm; MS (+CI, methane) m/z 447 $[M+1]^+$; M_r (+CI, methane) 447.223 35 $(M+1)^+$ (calcd for $C_{22}H_{31}N_4O_6$, 447.224 36).

7-*N*-(*cis*-2-Hydroxycyclohexyl)porfiromycins (25) and (26). Using **15** (8 mg, 22 μmol), **28** (14 mg, 92 μmol), TEA (14 μL, 103 μmol) and MeOH (2.5 mL) provided compounds (**25**) and (**26**) (1 d) as a solid.

Compound (25)[17] (2.5 mg, 25%): HPLC t_R: 25.1 min (instrument B); R_f 0.52 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 220, 369 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.22-1.39 (m, 1 H), 1.57-1.61 (m, 2 H), 1.65-1.72 (m, 4 H), 1.86-1.89 (m, 1 H), 1.96 (s, 3 H), 2.27 (s, 3 H), 2.28-2.31 (m, 2 H), 3.18 (s, 3 H), 3.47 (dd, J = 1.8, 12.9 Hz, 1 H), 3.57 (dd, J = 4.2, 10.8 Hz, 1 H), 3.94-4.02 (m, 2 H), 4.25 (d, J = 12.9 Hz, 1 H), 4.35 (dd, J = 10.5, 10.8 Hz, 1 H), 4.72 (dd, J = 4.2, 10.5 Hz, 1 H), 6.82 (d, J = 9.3 Hz, 1 H), the NMR sample contained triethylammonium hydrochloride: δ 1.25 (t, J = 7.2 Hz, 3 H) 3.72 (q, J = 7.2 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 10.0, 19.7, 23.7, 28.9, 32.1, 42.8, 43.0, 43.4, 46.4, 49.9, 50.0, 55.1, 62.7, 69.3, 104.0, 106.1, 110.0, 146.7, 155.3, 156.5, 176.4, 178.9 ppm; MS (+CI, methane) m/z 447 $[M+1]^+$; M_r (+CI, methane) 447.224 20 $(M+1)^+$ (calcd for $C_{22}H_{31}N_4O_6$, 447.224 36).

Compound (26)[17] (3.2 mg, 33%): HPLC t_R: 25.8 min (instrument B); R_f 0.47 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 220, 369 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24-1.38 (m, 1 H), 1.57-1.60 (m, 2 H), 1.66-1.71 (m, 4 H), 1.86-1.88 (m, 1 H), 1.97 (s, 3 H), 2.27 (s, 3 H), 2.29-2.33 (m, 2 H), 3.18 (s, 3 H), 3.47 (d, *J* = 13.2 Hz, 1 H), 3.57 (dd, *J* = 4.5, 10.8 Hz, 1 H), 3.92-3.98 (m, 2 H), 4.25 (d, *J* = 13.2 Hz, 1 H), 4.34 (dd, *J* = 10.5, 10.8 Hz, 1 H), 4.70 (dd, *J* = 4.5, 10.5 Hz, 1 H), 6.82 (d, *J* = 9.6 Hz, 1 H), the NMR sample contained triethylammonium hydrochloride: δ 1.25 (t, *J* = 6.9 Hz, 3 H), 3.73 (q, *J* = 6.9 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 10.0, 19.7, 23.8, 28.8, 32.2, 42.8, 43.0, 43.4, 46.4, 49.9, 50.0, 55.1, 62.7, 69.2, 104.0, 106.1, 110.0, 146.8, 155.4, 156.6, 176.4, 178.9 ppm; MS (+CI, methane) m/z 447 $[M+1]^+$; M_r (+CI, methane) 447.224 98 $(M+1)^+$ (calcd for $C_{22}H_{31}N_4O_6$, 447.224 36).

Synthesis of 31 and 32. To the methanolic solution (0.4 mL) of **15** (3.8 mg, 10 μmol) and **30** (2.7 μL, 5 μmol) were added TEA (1.5 μL, 10 μmol) and catalytic amount of K_2CO_3 (2 d). The reaction mixture provided **31** and **32** as solids.

Compound (31) (1 mg, 16%): HPLC t_R: 31.3-33.0 min (instrument B); R_f 0.21 (10% MeOH-$CHCl_3$); MS (+CI, methane) m/z 592 $[M+1]^+$; M_r (+CI, methane) 592.262 27 $[M+1]^+$ (calcd for $C_{28}H_{42}N_5O_5S_2$, 592.262 74).

Compound (32) (1.5 mg, 31%): HPLC t_R: 36.7 min (instrument B); R_f 0.43 (10% MeOH-$CHCl_3$); MS (+CI, methane) m/z 922 $[M+1]^+$; M_r (+CI, methane) 922.372 97 $[M+1]^+$ (calcd for $C_{44}H_{58}N_8O_{10}S_2$, 922.371 73).

Synthesis of C(7) Cyclized Porfiromycins (39) and (40). Using **15** (4.2 mg, 12 μmol), **38** (13 mg, 120 μmol) and MeOH (0.2 mL) gave **39** and **40** (2 d).

Compound (39)[17] (1.6 mg, 31%): HPLC t_R: 32.8 min (instrument A); R_f 0.63 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 236, 353 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 0.90-0.93 (m, 1 H), 1.23-1.35 (m, 3 H), 1.29 (d, *J* = 6.6 Hz, 3 H), 1.42-1.51 (m, 1 H), 1.58-1.63 (m, 1 H), 2.09 (dd, *J* = 1.8, 4.5 Hz, 1 H), 2.20 (s, 3 H), 2.50 (d, *J* = 4.5 Hz, 1 H), 2.69-2.72 (m, 1 H), 2.95-3.01 (m, 1 H), 3.03 (s, 3 H), 3.26 (q, *J* = 6.6 Hz, 1 H), 3.27-3.31 (m, 1 H), 3.38 (dd, *J* = 1.8, 12.6 Hz, 1 H), 3.62-3.68 (m, 1 H), 3.91 (dd, *J* = 4.5, 11.1 Hz, 1 H), 3.85 (d, *J* = 12.6 Hz, 1 H), 3.99 (dd, *J* = 6.3, 6.3 Hz, 1 H), 4.81 (dd, *J* = 10.5, 11.1 Hz, 1 H), 5.39 (dd, *J* = 4.5, 10.5 Hz, 1 H); ^{13}C NMR (pyridine-d_5, 150 MHz) 7.8, 23.8, 25.9, 28.7, 42.7 (2C), 44.1, 45.3, 47.0, 49.5, 49.7, 51.4, 58.4, 62.3, 73.2, 97.3, 106.4, 121.6, 156.6, 158.0, 191.7, 193.4 ppm; MS (+CI, methane) m/z 447 $[M+1]^+$; M_r (+CI, methane) 447.224 02 $(M+1)^+$ (calcd for $C_{22}H_{31}N_4O_6$ 447.224 36).

Compound (40) (0.6 mg, 12%): HPLC t_R: 30.6 min (instrument A); R_f 0.56 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 239, 365 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 0.95-0.98 (m, 1 H), 1.22-1.25 (m, 3 H), 1.32 (d, *J* = 6.6 Hz, 3 H), 1.46-1.50 (m, 1 H), 1.54-1.57 (m, 1 H), 2.04-2.08 (m, 1 H), 2.12 (br d, *J* = 4.5 Hz, 1 H), 2.20 (s, 3 H), 2.49-2.51 (m, 1 H), 2.54 (d, *J* = 4.5 Hz, 1 H), 3.12-3.16 (m, 1 H), 3.15 (s, 3 H),

3.41 (q, *J* = 6.6 Hz, 1 H), 3.44 (dd, *J* = 1.5, 12.3 Hz, 1 H), 3.46-3.49 (m, 1 H), 4.01 (dd, *J* = 4.5, 11.1 Hz, 1 H), 3.90 (d, *J* = 12.3 Hz, 1 H), 4.32 (dd, *J* = 6.0, 6.6 Hz, 1 H), 4.74 (dd, *J* = 10.5, 11.1 Hz, 1 H), 5.44 (dd, *J* = 4.5, 10.5 Hz, 1 H); ^{13}C NMR (pyridine-d_5, 150 MHz) 7.4, 23.4, 25.7, 28.1, 42.8, 42.9, 44.4, 46.9, 47.0, 49.5, 49.7, 52.5, 58.2, 62.2, 72.9, 98.3, 106.3, 121.9, 157.0, 158.0, 193.0 ppm, the remaining signal was not observed; MS (+CI, methane) m/z 447 $[M+1]^+$; M_r (+CI, methane) 447.223 49 $(M+1)^+$ (calcd for $C_{22}H_{31}N_4O_6$ 447.224 36).

7-*N*-(2-(*S*)-Hydroxypyrrolidine)porfiromycin (44). Using **15** (5.6 mg, 16 μmol), **42** (4.6 μL, 48 μmol) and MeOH (0.5 mL) provided **44** as a green solid (5.2 mg, 78%) (2 h): HPLC t_R: 24.1 min (instrument A); R_f 0.40 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 230, 379 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.50–1.54 (m, 1 H), 1.70-1.78 (m, 1 H), 1.84–1.89 (m, 1 H), 1.92 (s, 3 H), 2.02–2.08 (m, 1 H), 2.10 (d, *J* = 4.5 Hz, 1 H), 2.20 (s, 3 H), 2.50 (d, *J* = 4.5 Hz, 1 H), 3.10 (dd, *J* = 7.5, 11.4 Hz, 1 H), 3.13 (s, 3 H), 3.46 (d, *J* = 12.6 Hz, 1 H), 3.70-3.80 (m, 3 H), 3.92 (dd, *J* = 4.5, 11.1 Hz, 1 H), 4.46 (d, *J* = 12.6 Hz, 1 H), 4.77 (dd, *J* = 10.5, 11.1 Hz, 1 H), 5.18-5.25 (m, 1 H), 5.32 (dd, *J* = 4.5, 10.5 Hz, 1 H); ^{1}H NMR (CD_3OD, 600 MHz, 0 ºC) δ 1.71–1.75 (m, 1 H), 1.80-1.85 (m, 1 H), 1.86 (s, 3 H), 1.98-2.01 (m, 1 H), 2.10–2.15 (m, 1 H), 2.26 (s, 3 H), 2.44 (br s, 1 H), 2.51 (d, *J* = 4.6 Hz, 1 H), 3.19 (s, 3 H), 3.28-3.31 (m, 1 H), 3.41 (d, *J* = 12.9 Hz, 1 H), 3.42-3.49 (m, 2 H), 3.53 (dd, *J* = 4.3, 11.4 Hz, 1 H), 3.85-3.89 (m, 1 H), 4.15 (dd, *J* = 10.5, 11.4 Hz, 1 H), 4.20 (d, *J* = 12.9 Hz, 1 H), 4.64 (dd, *J* = 4.3, 10.5 Hz, 1 H), 4.91 (br s, 1 H); ^{13}C NMR (CD_3OD, 150 MHz, 0 ºC) 13.6, 26.7, 29.4, 43.2, 44.2, 45.0, 47.8, 50.0, 50.6, 57.4, 63.4, 63.5, 65.4, 107.1, 112.7, 114.7, 153.2, 153.8, 159.8, 181.1, 182.1 ppm; MS (+CI, methane) m/z 433 $[M+1]^+$; M_r (+CI, methane) 433.207 76 $(M+1)^+$ (calcd for $C_{21}H_{29}N_4O_6$, 433.208 71).

7-*N*-(2-(*R*)-Hydroxypyrrolidine)porfiromycin (45). Using **15** (3.2 mg, 9 μmol), **43** (2.6 μL, 27 μmol), and MeOH (0.5 mL) provided **45** as a green solid (3.1 mg, 80%) (0.3 h): HPLC t_R: 23.3 min (instrument A); R_f 0.41 (10% MeOH-$CHCl_3$); UV-vis (MeOH) λ_{max} 233, 371 nm; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 1.57–1.62 (m, 1 H), 1.74-1.80 (m, 1 H), 1.95–1.98 (m, 1 H), 2.03 (s, 3 H), 2.08–2.12 (m, 1 H), 2.13 (d, *J* = 4.5 Hz, 1 H), 2.21 (s, 3 H), 2.55 (d, *J* = 4.5 Hz, 1 H), 3.12 (dd, *J* = 8.1, 10.5 Hz, 1 H), 3.17 (s, 3 H), 3.52 (d, *J* = 12.6 Hz, 1 H), 3.77-3.86 (m, 3 H), 3.95 (dd, *J* = 4.4, 11.1 Hz, 1 H), 4.31 (d, *J* = 12.6 Hz, 1 H), 4.78 (dd, *J* = 10.5, 11.1 Hz, 1 H), 5.33-5.37 (m, 1 H), 5.53 (dd, *J* = 4.4, 10.5 Hz, 1 H); ^{1}H NMR (CD_3OD, 600 MHz, 0 ºC) δ 1.71–1.75 (m, 1 H), 1.81-1.83 (m, 1 H), 1.86 (s, 3 H), 1.98-2.00 (m, 1 H), 2.10–2.14 (m, 1 H), 2.25 (s, 3 H), 2.44 (br s, 1 H), 2.51 (d, *J* = 4.5 Hz, 1 H), 3.18 (s, 3 H), 3.33-3.36 (m, 1 H), 3.43 (dd, *J* = 0.9, 13.0 Hz, 1 H), 3.46-3.48 (m, 2 H), 3.53 (dd, *J* = 4.2, 11.4 Hz, 1 H), 3.85-3.89 (m, 1 H), 3.98 (d, *J* = 13.0 Hz, 1 H), 4.12 (dd, *J* = 10.6, 11.4 Hz, 1 H), 4.69 (dd, *J* = 4.2, 10.6 Hz, 1 H), 4.87 (br s, 1 H); ^{13}C NMR (CD_3OD, 150 MHz, 0 ºC) 13.1, 26.6, 29.2, 43.1, 43.9, 45.1, 48.1, 49.9, 50.8, 57.4, 63.1, 63.6, 65.1,

107.2, 112.9, 114.5, 153.8, 154.9, 159.6, 180.2, 182.2 ppm; MS (+CI, methane) m/z 433 $[M+1]^+$; M_r (+CI, methane) 433.209 17 $(M+1)^+$ (calcd for $C_{21}H_{29}N_4O_6$, 433.208 71).

Synthesis of 2-Acetylthiomethyl-1-[*tert*-butoxycarbonyl]piperidine (48). To a dry THF solution (3 mL) of triphenylphosphine (144 mg, 0.55 mmol) was added diethyl azodicarboxylate (DEAD) (86 μL, 0.55 mmol) at rt under Ar. The reaction solution was cooled in an ice bath and additional THF (1.5 mL) was added under Ar. After 20 min, a THF solution (1 mL) of **47**[23] (100 mg, 0.47 mmol) was added and then thiolacetic acid (39 μL, 0.55 mmol) was added slowly with stirring at 0 ºC. The reaction solution was allowed to stir at 0 ºC (1 h) and then at rt (16 h). The solvent was removed under low pressure and the residue was purified using PTLC (2% MeOH-$CHCl_3$) to give **48** as an oil (90 mg, 71%): R_f 0.72 (2% MeOH-$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38-1.41 (m, 1 H), 1.42 (s, 3 H), 1.57-1.64 (m, 5 H), 2.30 (s, 3 H), 2.73 (dd, *J* = 12.3, 13.2 Hz, 1 H), 3.04 (dd, *J* = 7.2, 13.5 Hz, 1 H), 3.14 (dd, *J* = 8.4, 13.5 Hz, 1 H), 3.96 (d, *J* = 13.2 Hz, 1 H), 4.25-4.30 (m, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 18.8, 25.2, 27.4, 28.4, 29.7, 30.5, 38.8, 49.5, 79.5, 154.9, 195.3 ppm; MS (+CI, methane) m/z 274 $[M+1]^+$; M_r (+CI, methane) 274. 147 72 $(M+1)^+$ (calcd for $C_{13}H_{24}NO_3S$ 274.147 69).

Synthesis of 2-Mercaptomethyl-1-[*tert*-butoxycarbonyl]piperidine (49). To a MeOH/H_2O solution (2:1, 3 mL) of **48** (23 mg, 0.08 mmol) was added K_2CO_3 (23 mg, 0.17 mmol) and the reaction mixture was stirred at rt (25 min). The MeOH was removed under reduced pressure and residue was extracted with CH_2Cl_2 (15 mL). The organic layer was dried (Na_2SO_4) and solvent was removed *in vacuo* to give crude **49** as an oil (20 mg, 93%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30-1.38 (m, 2 H), 1.45 (s, 9 H), 1.52-1.62 (m, 3 H), 1.86 (d, *J* = 13.5 Hz, 1 H), 2.59 (dd, *J* = 8.4, 13.5 Hz, 1 H), 2.63-2.66 (m, 1 H), 2.78 (dd, *J* = 7.5, 13.5 Hz, 1 H), 3.98 (d, *J* = 12.9 Hz, 1 H), 4.18-4.22 (m, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 18.7, 24.5, 25.2, 26.3, 28.4, 39.1, 52.9, 79.5, 155.1 ppm; MS (+CI, methane) m/z 232 $[M+1]^+$; M_r (+CI, methane) 232.135 85 $(M+1)^+$ (calcd for $C_{11}H_{22}NO_2S$ 232.137 13).

Synthesis of 2-(Mercaptomethyl)piperidine·HI (46). To a $CDCl_3$ solution (1 mL) of **49** (8 mg, 35 μmol) was added trimethylsilyl iodide (6.1 μL, 55 μmol) under Ar. The reaction solution was stirred at rt (20 min) and then MeOH (1 mL) was added, and the reaction solution was stirred for additional 17 h. The solvent was removed *in vacuo* to give crude **46** as an oil (9 mg, 100%): ^{1}H NMR (D_2O, 300 MHz) δ 1.47-1.65 (m, 3 H), 1.87-1.90 (m, 2 H), 2.00 (d, *J* = 13.5 Hz, 1 H), 2.68 (dd, *J* = 7.2, 15.0 Hz, 1 H), 2.90 (dd, *J* = 5.1, 15.0 Hz, 1 H), 2.96-3.05 (m, 1 H), 3.20-3.22 (m, 1 H), 3.44 (d, *J* = 13.2 Hz, 1 H); ^{13}C NMR (D_2O, 75 MHz) 21.4, 21.8, 26.6, 27.5, 45.0, 58.5 ppm; MS (+CI, methane) m/z 132 $[(M-HI)+1]^+$.

Reaction of 15 with 2-(Mercaptomethyl)piperidine·HI (46). To a dry methanolic solution (0.6 mL) of **15** (4.5 mg, 12 μmol) and **46** (6.4 mg, 24 μmol) were added TEA (3.4 μL, 24 μmol) and a catalytic amount of K_2CO_3 under Ar. The reaction mixture was stirred at rt under Ar. After 15 min the color of

reaction mixture changed from purple to orange and an orange color solid precipitated from solution. The solvent was removed *in vacuo* under low pressure and the residue was purified by PTLC (10% MeOH-$CHCl_3$) to give **50** (1.5 mg, 37%) as an orange solid (HPLC t_R 22.5 min, R_f 0.42 (10% MeOH-$CHCl_3$)). The separated solid was coinjected with authentic **50**[24] and showed a single peak at 22.5 min.

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