Tetrahedron: *Asymmetry* 21 (2010) 2625–2630

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

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

Asymmetric syntheses of piperidino-benzodiazepines through 'cation-pool' host/guest supramolecular approach and their DNA-binding studies

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ARTICLE INFO

Article history:
Received 10 August 2010
Accepted 27 October 2010
Available online 20 November 2010

ABSTRACT

The asymmetric synthetic approach to piperidino-benzodiazepine **4a** (a homolog of DC-81) has been developed. The absolute stereochemistry of **4** and **5** has been assigned to be (*S*) at C-12a position. This procedure features the use of a 'cation-pool' strategy and also a host/guest supramolecular co-catalysis approach. In this study, the chloroformate of 8-phenylmenthyl has been employed as a chiral auxiliary and includes one-pot conditions for anodic oxidation, which are followed by nucleophilic addition to an *N*-acyliminium ion. In addition, intramolecular azido reductive-cyclization and nitro reductive dithioacetal deprotective tandem-cyclization approaches have also been utilized for the syntheses of these compounds **4a**,**b** and **5a**,**b**. Some of the representative compounds exhibited an enhanced DNA-binding ability in comparison to the natural product DC-81.

1. Introduction

A growing interest in the development of new synthetic routes for the construction of chiral *N*-heterocycles continues to be essential for accessing natural and unnatural products, particularly in a stereoselective manner. Nowadays, chirality in organic molecules plays an enormous role in areas ranging from medicine to material science, yet the synthesis of such entities in one enantiomeric form is considered as one of the most difficult challenges that need to be addressed by the synthetic chemists. However, inspite of many developments in synthetic organic chemistry, still there is scope for the development of newer methods that could provide important insights in employing chiral auxiliaries for the stereoselective construction of predetermined moieties in certain classes of compounds. As part of our efforts in the field of biologically relevant *N*-moieties, we became interested in the development of a new asymmetric synthetic route for the preparation of imine-containing piperidino-benzodiazepines and their dilactams.

There has been increasing interest in the synthesis of DNA sequence selective binding agents, particularly by low molecular weight antitumor antibiotics. Among them, the pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a well known class of DNA-interactive potent antitumor agents derived from *Streptomycin* species.

Streptomycin species,[1,2] which include anthramycin **1**, tomaymycin **2,** and DC-81 **3** (Fig. 1). A stereogenic center at the C11a position and a DNA-reactive imine moiety at the N10–C11 position, are characteristics of these tricyclic PBD's that have been extensively investigated and rationalized to obtain a snug fit into the minor groove of duplex DNA. The interaction of PBD with DNA is due to a covalent aminal bond with the N2–amino group of the guanine base[3] that provides a preference for Pu–G–Pu sequences.[1] Interestingly, these compounds have also been shown to inhibit both endonuclease[4] and RNA polymerase[5] enzymes in a sequence-dependent manner. A large number of PBDs have been designed and synthesized with a view to understand their structure–activity relationship[6–8] apart from the development of a variety of synthetic approaches[9,10] both in solution-phase and on the solid-phase.[11,12]

Based on this analogy we have replaced the pyrrole (five-membered ring) with piperidine (six-membered ring) to evaluate their potential for DNA-binding interactions with duplex DNA by modifying the stereogenic position from C11a to C12a. Herein, we have developed a new asymmetric synthetic route for the piperidino-benzodiazepines (PiBDs) through a β-CD-host/guest supramolecular approach using 8-phenylmenthyl chloroformate as a chiral auxiliary for the construction of the (*S*)-pipecolic moiety. Takaya et al.[13] have reported the synthesis of racemic (±)-piperidino-benzodiazepine dilactam **5a** from D,L-pipecolic acid. However, only a few methods have been reported for the synthesis of optically active pipecolic acid derivatives, mainly involving enzymatic routes,[14] alkylation of chiral glycine enolates,[15] from natural amino

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doi:10.1016/j.tetasy.2010.10.030

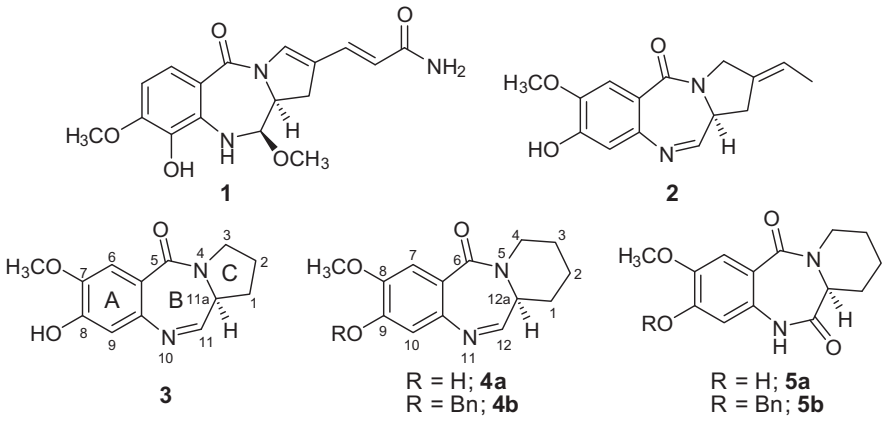


Figure 1. Representative chemical structures of anthramycin **1**, tomaymycin **2**, DC-81 **3**, a homolog of DC-81 **4a**, and its derivatives **4b** and **5a**,**b**.

acids[16], and other enantioselective synthetic processes.[17] Recently, we developed a synthetic strategy that was based on the diastereoselective addition of NC^- to an *N*-acyliminium ion bearing a chiral auxiliary (8-phenylmenthyl) for the introduction of asymmetry into pipecolic acid.[18] In this context, we became interested in applying this protocol to the synthesis of optically active PiBDs and to evaluate their DNA-binding ability.

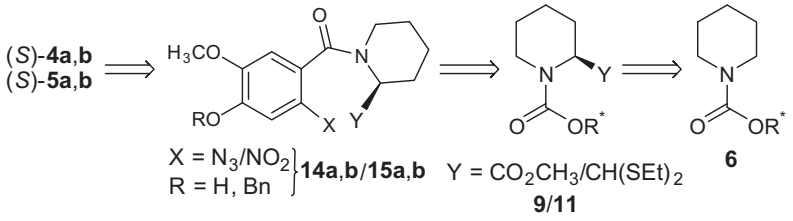
2. Results and discussion

The synthetic strategy for the preparation of compounds **4a**,**b** and **5a**,**b** is outlined in Scheme 1. The key intermediates **10**/**12** were synthesized from L-pipecolic acid, which was obtained from the *N*-carbamate of 8-phenylmenthyl piperidine **6** by anodic oxidation using a 'cation-pool' technique. Next, we attempted to construct the tricyclic framework based on two approaches: (i) a nitro reduction followed by diethanethiol deprotective-cyclization[19] for the synthesis of PiBD imines **4a**,**b**; and (ii) azido reductive tandem-cyclization[20] for PiBD dilactams **5a**,**b**. We also attempted the reduction of azido ester **15a** to the corresponding aldehyde with DIBAL-H, however, this reaction produced a complicated mixture even at very low temperatures (−78 °C).

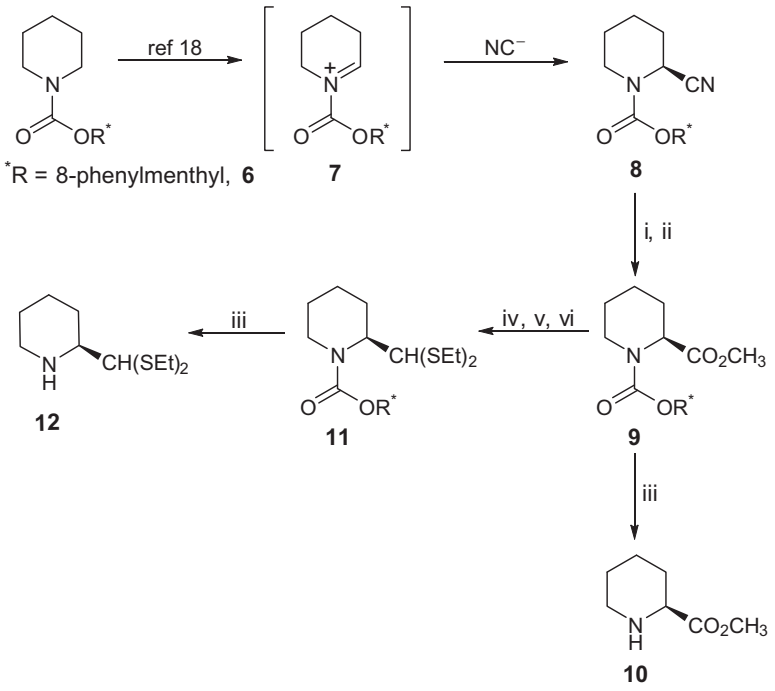
According to our previous model experiments,[18] the best conditions were obtained by nucleophilic addition of TMSCN in the presence of β-CD to *N*-acyliminium ion **6** by using TMSOTf as a Lewis acid. Furthermore, the use of the chloroformate of 8-phenylmenthyl as a chiral auxiliary affords the corresponding α-methoxycarbamate, which reacts in situ with TMSCN catalyzed by TMSOTf in CH_2Cl_2 at −40 °C. The protocol provided **8** in 65% yields and 91% de. The diastereomeric excess was determined by chiral HPLC analysis using a ChiralPack OD column and the enantiomeric excess was determined after hydrolysis of **8** as (*S*)-(−)-pipecolic acid, $[\alpha]_D^{31} = -26$ (*c* 1.0, H_2O), mp: 271–272 °C (lit. mp: 272 °C). Compound **8** was hydrolyzed with 1 M HCl and taken up for further esterification with $SOCl_2$ in MeOH to give **9** in 80% yield. Next, we determined the synthesis of dithioacetal protected key intermediate **11** from **9** as depicted in Scheme 2.

The selective reduction of ester **9** to the corresponding aldehyde employing DIBAL-H in CH_2Cl_2 at −78 °C for 45 min did not proceed well. Therefore, the ester group was reduced completely to the alcohol (80%), and the aldehyde was obtained in very low yield (10%). Moreover, the remaining starting material **9** was recovered (~10%) and the reaction was monitored by GC analysis. We decided to employ the Dess–Martin periodinate for the oxidation of the alcohol to an aldehyde. It was observed that the ester reduction followed by periodinate oxidation afforded the aldehyde in excellent yield (92%). The aldehyde (without purification) was immediately protected with EtSH/TMSCl in CH_2Cl_2 at room temperature for 6 h to give **11** in 95% yields. Finally, the chiral intermediates **10**/**12** were obtained by the removal of the chiral auxiliary using HCl/$CHCl_3$ (6.0 M) at reflux for 48 h. These intermediates are directly utilized for the next step without any further purification. It should be noted that when $CHCl_3$ was used as the solvent with no excess of water, ester **10** was isolated as its hydrochloride salt accompanied by the chiral auxiliary (95%). When the reaction was performed in H_2O, pipecolic acid was obtained in 99% yield. The enantiomeric excess of compounds **10** and **12** were determined from the phenylamide derivatives of **10** and **12**, which were subjected to HPLC analysis by employing a chiral Welch-01 column (*n*-hexane/isopropanol = 9:1, 1.0 mL/min, 254 nm UV detector), the enantiomeric excess for **10** and **12** were determined as >95%.

The substituted azido benzoic acids **13a**,**b** were coupled with methyl (2*S*)-piperidinoate **10** using EDCI/HOBt in CH_2Cl_2 by stirring them overnight to provide **15a**,**b** in good yields (75% and 86%, respectively). Another protocol was also employed for the coupling of nitro acids with (2*S*)-piperidino-2-carboxaldehyde diethyl dithioacetal **12**. The nitro acids were initially treated with $SOCl_2$ in dry benzene with a catalytic amount of DMF (added for solubility). Then, after evaporation it was added dropwise to a stirred solution of **12** employing Et_3N as a base taken in anhydrous THF



Scheme 1. Retrosynthetic analysis for piperidino-benzodiazepines (**4a**,**b** and **5a**,**b**).

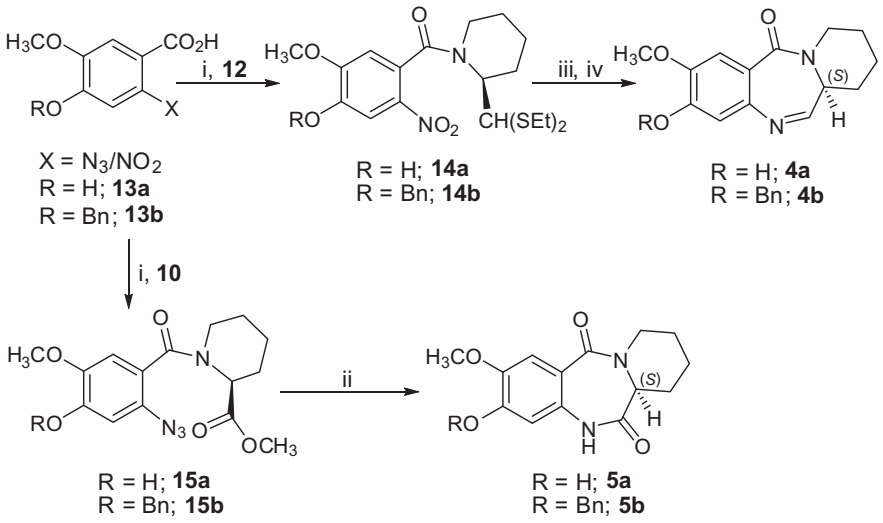


Scheme 2. Reagents and conditions: (i) 1 M HCl, rt, 5 h; (ii) $SOCl_2$, MeOH, rt, 12 h, 80% (two steps); (iii) 6 M HCl/$CHCl_3$, 48 h, reflux; (iv) DIBAL-H, CH_2Cl_2, −78 °C, 45 min, 80%; (v) Dess–Martin periodinate, rt, 30 min, 92%; (vi) TMSCl/EtSH, CH_2Cl_2, rt, 6 h, 95%.

at 0 °C in about 30 min. The reaction mixture was then brought to ambient temperature and stirred for another 2 h to give **14a**,**b** in 88% yield. The reduction of nitro dithioacetal intermediates **14a**,**b** with $SnCl_2{\cdot}2H_2O$ followed by deprotective-cyclization using $HgCl_2/CaCO_3$ in CH_3CN/H_2O (4:1) affords the piperidino-benzodiazepines **4a**,**b**. Compound **4a** was obtained in 68% overall yield from these two steps, and no racemization was observed in the process, $[\alpha]_D^{31} = +256$ (*c* 1.0, $CHCl_3$), mp: 119–121 °C; (natural product DC-81) $[\alpha]_D = +135$ (*c* 0.2, $CHCl_3$). Analogously, compound **4b** was also obtained in 75% yield, $[\alpha]_D^{31} = +295$ (*c* 1.0 in $CHCl_3$); mp: 69–71 °C. All the compounds were characterized by NMR and MS spectroscopic studies. The specific rotation values for these final compounds suggest that the stereochemistry at (*S*)-C12a is retained throughout the synthesis. We also carried out an intramolecular azido reductive tandem-cyclization process employing $Al(OTf)_3$/NaI for the reduction of azides **15a**,**b**. It was observed that the substituted 2-azidobenzoyl piperidine esters **15a**,**b** reduced selectively with $Al(OTf)_3$ (20 mol %) and NaI (3 equiv) by using CH_3CN as a solvent to provide the desired dilactams **5a**,**b** in good yields (85–88%) as shown in Scheme 3.

3. DNA-binding ability studies

The DNA-binding ability of PiBDs **4a**,**b** was examined by thermal denaturation studies using calf-thymus (CT) DNA.[21] Melting studies showed that these compounds stabilize the thermal he-



Scheme 3. Reagents and conditions: (i) EDCI/HOBt, CH_2Cl_2, 0 °C to rt, overnight, 75–86% for azides and (a) $SOCl_2$, benzene, 1–2 drops DMF, 6 h, (b) Et_3N, dry THF, 0 °C to rt, 2 h, 88–90% for nitro group; (ii) $Al(OTf)_3$/NaI, CH_3CN, 20 min, 85–88% for azides; (iii) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 5 h for nitro group; (iv) $HgCl_2$–$CaCO_3$, CH_3CN/H_2O (4:1), 6 h, 68–75% (two steps overall yield).

Table 1
Thermal denaturation data for piperidino-benzodiazepines **4a**,**b** and **5a**,**b** with calf thymus CT-DNA

Compounds	[PiBD]/[DNA] molar ratio[a]	ΔT_m[b] (°C) after incubation at 37 °C for	
		0 h	18 h
4a	1:5	1.2	2.2
4b	1:5	0.9	1.3
5a	1:5	1.2	1.8
5b	1:5	0.4	0.6
DC-81 (3)	1:5	0.3	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.6 °C ± 0.01 (mean value from eight separate determinations), all T_m values are ±0.05 to 0.15 °C.
[b] For a 1:5 molar ratio of [PiBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

lix-coil or melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0, incubated at 37 °C, where PiBD/DNA molar ratio was 1:5. These studies show the melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0, incubated at 37 °C, where the PiBD/DNA molar ratio was 1:5. An increase in helix melting temperature (ΔT_m) up to 2.2 °C for **4a** was observed compared to an untreated control DNA after 18 h incubation at 37 °C. However, in the same experiment, the natural product DC-81 **3** exhibited a ΔT_m of 0.7 °C, consistent with the notion that the homolog of DC-81 **4a** forms a more stable DNA-binding complex by replacing the (five-membered) pyrrole with a piperidine ring (six-membered ring). Analogously, compound **4b** elevates the helix melting temperature of CT-DNA by 0.9 °C at 0 h, which did not show a significant difference in the melting temperatures even after incubation for 18 h (1.3 °C). Furthermore, the non-covalent DNA-interactive PiBD dilactams **5a**,**b** were also evaluated for their DNA-binding ability by using a similar procedure. The dilactams **5a**,**b** exhibited moderate binding to the DNA as ΔT_m values ranged from 0.4 to 1.8 °C. These results are illustrated in Table 1.

4. Conclusion

In conclusion, we have reported a facile asymmetric synthetic route to an important class of piperidino-benzodiazepines from commercially available piperidine and easily accessible aromatic azido/nitro acid derivatives. In this protocol, we employed a one-pot procedure for the generation of the required stereogenic center in the *N*-acyliminum ion by anodic oxidation through a 'cation-pool' technique using host/guest supramolecular diasteroselective approach, with significantly improved % de. Furthermore, an intramolecular azido reductive-cyclization and dithioacetal deprotection tandem-cyclization approach has also been applied to the synthesis of these heterocycles. Interestingly, some of these molecules exhibit an enhanced DNA-binding ability in comparison to DC-81. This new strategy provides potential applications for the syntheses of both natural as well as medicinally important heterocyclic compounds.

5. Experimental

5.1. General methods

Purchased chemical reagents were used without further purification. Anhydrous THF, CH_2Cl_2, CH_3CN, MeOH, and DMF were prepared by distillation under a nitrogen atmosphere over sodium/benzophenone, CaH_2, sodium/P_2O_5, and CaH_2/molecular sieves, respectively, and were used for reactions. Solvents for extraction and column chromatography were distilled prior to use. Sodium azide was handled with care for the preparation of substituted 2-azidobenzoic acids by wearing safety glasses; facemask, gloves, and reactions were performed in a fume hood. IR spectroscopy, FT-IR Nicolet Nexus 470 equipment and KCl cell. Infrared spectra were recorded and the wave numbers are expressed in cm^{-1}. Melting points (uncorrected) were measured with an Electrothermal apparatus. Platinum plate anode (4.0 cm^2) and tungsten wire as cathode was used for the 'cation-pool' technique. Thermal denaturation (DNA-binding) studies were evaluated by BECKMAN COULTER-DU 800 spectrophotometer. Specific rotations were recorded on SEPA-300 (Horiba high sensitive polarimeter) fixed with a sodium lamp of wavelength 589 nm. ^1H and ^{13}C NMR spectra were recorded on Gemini 200, Avance 300, Inova 400, Inova 500, and Bruker 600 MHz spectrometers using tetramethyl silane (TMS) as the internal standard. Chemical shifts are reported in parts per million (ppm) downfield from tetramethyl silane. Spin multiplicities are described as s (singlet), br s (broad singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and or m (multiplet). Coupling constants are reported in Hertz (Hz). Mass spectra were recorded on a Quattro-LC, (ESI). Column chromatography was performed using silica gel 60–120 and 100–200 mesh. TLC analyses were performed with silica gel plates using iodine, $KMnO_4$ and UV-lamp for visualization.

5.1.1. (*S*)-2-Methyl 1-((1*R*,2*S*,5*R*)-5-methyl-2-(2-phenylpropan-2-yl)cyclohexyl) piperidine-1,2-dicarboxylate 9

White crystalline solid, mp: 60–62 °C; $[\alpha]_D^{31} = -49.9$ (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.27 (m, 4H), 7.13 (t, 1H, *J* = 5.84, 6.57 Hz), 4.71–4.78 (dd, 1H, *J* = 3.65, 4.38 Hz), 3.94 (dt, 1H, *J* = 2.19, 11.68 Hz), 3.63 (s, 3H), 3.36 (d, 1H, *J* = 5.11 Hz), 2.72 (t, 1H, *J* = 9.49, 10.95 Hz), 1.95–2.04 (m, 2H), 1.85–1.88 (m, 3H), 1.77 (m, 1H), 1.55–1.67 (m 6H), 1.17 (s, 3H), 0.95 (m, 2H), 0.84 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 152.4, 127.8, 127. 6, 125.0, 124.8, 124.6, 54.0, 53.6, 51.6, 50.7, 50.5, 42.2, 41.5, 39.2, 34.5, 31.1, 29.2, 26.9, 26.4, 24.6, 24.3, 23.1, 21.7, 20.7. (ESI) HRMS: *m*/*z* calcd for $C_{24}H_{35}NO_4Na$ 424.2463, found 424.2452 $[M]^{+\cdot}$.

5.1.2. (*S*)-((1*R*,2*S*,5*R*)-5-Methyl-2-(2-phenylpropan-2-yl)cyclohexyl) 2-(bis(ethylthio)methyl)piperidine-1-carboxylate 11

Diisobutylaluminiumhydride (DIBAL-H) solution (0.91 mL of 1.0 M solution in hexane) was added dropwise to a vigorously stirred solution of the compound **9** (230 mg, 0.573 mmol) in anhydrous CH_2Cl_2 (5 mL) under dry nitrogen at −78 °C. Next, the reaction mixture was stirred for an additional 30 min, unfortunately the ester group was reduced completely to the alcohol (171 mg, 80%), and the aldehyde obtained in low yield (10%). Moreover, the starting material **9** was recovered in around 10%. The following reaction was analyzed by GC analysis, a ratio of 8:1:1 from alcohol/aldehyde/**9** was observed. Next, the obtained corresponding alcohol (110 mg, 0.295 mmol) in CH_2Cl_2 (3 mL) was treated with Dess–Martin periodinate (150 mg, 0.353 mmol) oxidation to produce the aldehyde in excellent yield (100 mg, 92%). This reaction mixture was quenched with saturated aqueous solutions of $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) and stirred for another 15 min. The organic layer was separated, washed with brine, and then dried in anhydrous Na_2SO_4 followed by evaporation under reduced pressure. The aldehyde (100 mg, 0.269 mmol) was promptly (without column chromatographic purification) protected with EtSH (0.05 mL, 0.673 mmol), TMSCl (0.085 mL, 0.673 mmol) in CH_2Cl_2 at room temperature for 6 h to give **11** (122 mg) in 95% yield as a colorless liquid. $[\alpha]_D^{32} = -85.7$ (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.28 (m, 4H), 7.11 (t, 1H, *J* = 4.34, 6.98 Hz), 4.66–4.75 (ddd, 1H, *J* = 3.77, 3.96 Hz), 4.37 (d, 1H, *J* = 10.19 Hz), 4.05–4.17 (dd, 1H, *J* = 9.82, 10.57 Hz), 3.67 (m, 1H), 3.29 (d, 1H, *J* = 10.57 Hz), 2.51–2.82 (m, 5H), 2.53 (t, 2H, *J* = 11.27, 13.03 Hz), 2.27 (d, 1H, *J* = 12.08 Hz), 1.92–2.10 (m, 2H), 1.58 (s, 3H), 1.20–1.33 (m, 13H), 0.90–1.07 (m, 1H), 0.86 (s, 3H), 0.84 (s, 3H). ^{13}C

NMR (75 MHz, $CDCl_3$): δ 151.8, 127.9, 125.5, 125.2, 125.0, 124.6, 52.9, 52.3, 50.6, 42.3, 39.8, 39.0, 34.6, 31.2, 27.2, 26.9, 26.3, 25.8, 24.7, 23.7, 21.7, 18.9, 14.4. (ESI) HRMS: *m*/*z* calcd for $C_{27}H_{43}NO_2S_2$-Na 500.2632, found 500.2622 $[M]^{+\cdot}$.

5.1.3. (*S*)-(2-(Bis(ethylthio)methyl)piperidin-1-yl)(4-hydroxy-5-methoxy-2-nitrophenyl)methanone 14a

To a stirred solution of 4-hydroxy-5-methoxy-2-nitrobenzoic acid **13a** (300 mg, 1.408 mmol) and thionyl chloride (0.30 mL, 4.22 mmol) in dry benzene (10 mL), 1–2 drops of DMF were added and the stirring was continued for 6 h. Then, the benzene was evaporated in vacuo. The resultant oil was dissolved in dry THF (10 mL), and then added dropwise over a period of 30 min to a stirred suspension of (2*S*)-piperidino-2-carboxaldehyde diethyl dithioacetal **12** (308 mg, 1.408 mmol), Et_3N (0.60 mL, 4.224 mmol), and THF (5 mL) cooled in an ice bath. After the completion of addition, the reaction mixture was brought to ambient temperature and stirred for an additional 2 h. Next, the THF solvent was evaporated, the aqueous phase adjusted to pH 4 using 4 M HCl, and then extracted with ethyl acetate followed by washing with brine and dried over Na_2SO_4. This was further purified by column chromatography (80% EtOAc–hexane) to afford compound **14a** (364 mg, 88%) as a yellowish liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 1H), 6.98 (s, 1H), 6.05 (br s, 1H), 4.71 (d, 1H, *J* = 12.41 Hz), 4.27–4.35 (m, 1H), 3.97 (s, 3H), 3.20 (d, 1H, *J* = 14.18 Hz), 2.97–3.03 (m, 1H), 2.69–2.83 (m, 4H), 2.34 (t, 4H, *J* = 7.09 Hz), 1.52–1.71 (m, 2H), 1.25 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 167.2, 152.3, 146.0, 137.6, 126.3, 111.2, 108.9, 58.2, 56.5, 51.7, 50.2, 43.7, 38.2, 26.2, 25.1, 23.4, 18.9, 14.2. (ESI) MS: *m*/*z* 415 $[M]^{+\cdot}$.

5.1.4. (*S*)-(4-(Benzyloxy)-5-methoxy-2-nitrophenyl)(2-(bis(ethylthio)methyl)piperidin-1-yl)methanone 14b

Yield (333 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 1H), 7.34–7.46 (m, 5H), 6.99 (s, 1H), 5.20 (s, 2H), 4.98–5.05 (m, 1H), 4.26–4.34 (m, 1H), 3.95 (s, 3H), 3.30 (br s, 1H), 3.21 (d, 1H, *J* = 15.53 Hz), 2.91–3.00 (m, 2H), 2.68–2.82 (m, 4H), 2.31–2.47 (m, 1H), 4.54 (m, 1H), 1.52–1.70 (m, 2H), 1.25 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.5, 154.5, 147.7, 137.3, 135.3, 128.7, 128.4, 127.4, 111.6, 109.5, 109.0, 71.2, 58.0, 56.4, 51.8, 49.9, 43.6, 38.0, 29.6, 26.3, 25.6, 23.3, 22.1, 19.4, 14.3. EI-MS: *m*/*z* 505 $[M]^{+\cdot}$.

5.1.5. (*S*)-3-Hydroxy-2-methoxy-7,8,9,10-tetrahydrobenzo[*e*]-pyrido[1,2-*a*][1,4]diazepin-12(6a*H*)-one 4a (homolog of DC-81)

Compound **14a** (80 mg, 0.193 mmol) was dissolved in MeOH (5 mL). Next $SnCl_2{\cdot}2H_2O$ (217 mg, 0.966 mmol) was added and the mixture refluxed for 5 h or until TLC indicated that the reaction was complete. The methanol was evaporated by vacuum and the aqueous layer was then carefully adjusted to pH 8 with 10% $NaHCO_3$ solution and the tin salts separated through a Celite bed. Extraction was carried out with ethyl acetate (3 × 20 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the amino diethyl thioacetal. Next, this reaction mixture (70 mg, 0.182 mmol) was dissolved in CH_3CN/H_2O (4:1, 10 mL), after which $HgCl_2$ (123 mg, 0.455 mmol) and $CaCO_3$ (45 mg, 0.455 mmol) were added and stirred at room temperature for 6 h until TLC (ethyl acetate) indicated the complete loss of the starting material. The reaction mixture was further diluted with EtOAc (10 mL) and filtered through a Celite bed. The clear yellow organic supernatant was extracted with saturated 5% $NaHCO_3$ (10 mL) and the combined organic phase was dried over Na_2SO_4. The organic layer was evaporated in vacuo and purified by column chromatography (95% EtOAc–MeOH) affords the compound **4a** (32 mg, 68%). Mp: 119–121 °C, $[\alpha]_D^{31} = +256$ (*c* 1.0, $CHCl_3$); (natural product DC-81) $[\alpha]_D = +135$ (*c* 0.2, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.92 (d, 1H, *J* = 6.07 Hz), 7.39 (s, 1H), 7.25 (s, 1H), 5.29 (br s, 1H), 4.19–4.23 (m, 1H), 3.93 (s, 3H), 3.73–3.75 (m, 1H), 3.20–3.27 (m, 1H), 2.07–2.10 (m, 1H), 1.93–2.00 (m, 1H), 1.82–1.87 (m, 3H), 1.67–1.73 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.4, 162.5, 150.2, 145.6, 142.2, 135.2, 121.9, 112.0, 56.41, 49.8, 39.9, 24.4, 22.9, 18.2. (ESI) HRMS: *m*/*z* calcd for $C_{14}H_{16}N_2O_3$ 261.1239, found 261.1234 $[M+H]^{+\cdot}$.

5.1.6. (*S*)-3-(Benzyloxy)-2-methoxy-7,8,9,10-tetrahydrobenzo-[*e*]pyrido[1,2-*a*][1,4]diazepin-12(6a*H*)-one 4b

Compound **14b** (65 mg, 0.129 mmol) dissolved in MeOH (5 mL). Next, $SnCl_2{\cdot}2H_2O$ (145 mg, 0.645 mmol) was added and the mixture refluxed for 6 h or until TLC indicated that the reaction was complete. The methanol was evaporated by vacuum and the aqueous layer was then carefully adjusted to pH 8 with 10% $NaHCO_3$ solution and the tin salts separated through a Celite bed. Extraction was carried out with ethyl acetate (3 × 20 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the amino diethyl thioacetal. Next, the reaction mixture (59 mg, 0.124 mmol) was dissolved in CH_3CN/H_2O (4:1, 10 mL), after which $HgCl_2$ (84 mg, 0.311 mmol), and $CaCO_3$ (31 mg, 0.311 mmol) were added and stirred at room temperature for 6 h until TLC (ethyl acetate) indicated the complete loss of starting material. The reaction mixture was further diluted with EtOAc (10 mL) and filtered through a Celite bed. The clear yellow organic supernatant was extracted with saturated 5% $NaHCO_3$ (10 mL) and the combined organic phase was dried over Na_2SO_4. The organic layer was evaporated in vacuo and purified by column chromatography to afford compound **4b** (column chromatography, EtOAc 100%, 32 mg, 75%). Mp: 69–71 °C, $[\alpha]_D^{31} = +295$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, 1H, *J* = 5.93 Hz), 7.30–7.45 (m, 6H), 6.82 (s, 1H), 5.18 (m, 2H), 4.23 (d, 1H, *J* = 14.41 Hz), 3.92 (s, 3H), 3.74 (q, 2H, *J* = 7.28 Hz), 3.18–3.25 (m, 1H), 2.04–2.09 (m, 1H), 1.35–1.99 (m, 1H), 1.27 (t, 2H, *J* = 7.63 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 167.4, 163.0, 150.4, 148.1, 139.7, 136.1, 128.6, 128.0, 127.2, 121.4, 111.6, 110.6, 70.7, 56.1, 49.5, 39.6, 24.4, 22.9, 18.3. (ESI) HRMS: *m*/*z* calcd for $C_{21}H_{22}N_2O_3Na$ 373.1528, found 373.1547 $[M]^{+\cdot}$.

5.1.7. (*S*)-Methyl 1-(2-azido-4-hydroxy-5-methoxybenzoyl)-piperidine-2-carboxylate 15a

To a stirred solution of 4-hydroxy-5-methoxy-2-azidobenzoic acid **13a** (146 mg, 0699 mmol) in CH_2Cl_2 (5 mL), EDCI (200 mg, 1.048 mmol), and HOBt (141 mg, 1.048 mmol) were added and stirred for 30 min at 0 °C. Methyl (2*S*)-piperidinoate **10** (100 mg, 0.699 mmol) in CH_2Cl_2 (5 mL) was then added to the reaction mixture and stirring was continued at room temperature overnight. The reaction mixture was isolated in CH_2Cl_2 (3 × 20 mL), washed with $NaHCO_3$ solution (1 × 20 mL), brine (1 × 20 mL), and then dried over anhydrous Na_2SO_4. The crude product was further purified through column chromatography using (silica-gel 60–120 mesh) EtOAc–hexane (8:2) as eluent and the pure product **15a** (174 mg) was obtained in 75% yield. FT-IR: (cm^{-1}) 3455, 2943, 2860, 2111, 1739, 1637, 1511, 1428, 1393, 1363, 1247, 1210, 1168, 1072, 1014, 860, 814, 747, 698, 637. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.79 (s, 1H), 6.75 (s, 1H), 5.98 (br s, 1H), 5.51 (d, 1H, *J* = 6.04 Hz), 3.88 (s, 3H), 3.78 (s, 3H), 3.39–3.43 (m, 1H), 3.10–3.19 (m, 1H), 2.20–2.36 (m, 2H), 1.55–1.82 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.1, 168.4, 147.7, 144.5, 128.9, 118.7, 110.2, 105.2, 56.1, 52.2, 45.4, 39.7, 26.4, 25.1, 20.9. (ESI) HRMS: *m*/*z* calcd for $C_{15}H_{18}N_4O_5Na$ 357.1174, found 357.1164 $[M]^{+\cdot}$.

5.1.8. (*S*)-Methyl 1-(2-azido-4-(benzyloxy)-5-methoxybenzoyl)-piperidine-2-carboxylate 15b

To a stirred solution of 4-benzyloxy-5-methoxy-2-azidobenzoic acid **13b** (229 mg, 0.769 mmol) in CH_2Cl_2 (10 mL), EDCI (200 mg, 1.048 mmol), and HOBt (141 mg, 1.048 mmol) were added and stirred for 30 min at 0 °C. Methyl (2*S*)-piperidinoate **10** (100 mg,

0.699 mmol) in CH_2Cl_2 (5 mL) was added to the reaction mixture at the same temperature and stirring was continued at room temperature overnight. The crude product was further purified through column chromatography using (silica-gel 60–120 mesh) EtOAc–hexane (3:7) as eluent and the pure product **15b** (278 mg) was obtained in 86% yield. FT-IR: (cm^{-1}) 2946, 2859, 2114, 1740, 1617, 1516, 1436, 1312, 1256, 1212, 1168, 1073, 1044, 1010, 930, 866, 809, 755, 639. ^1H NMR (400 MHz, $CDCl_3$): δ 7.33–7.45 (m, 5H), 6.78 (m, 1H), 6.66 (m, 1H), 5.51 (s, 2H), 4.67 (d, 1H, J = 13.5 Hz), 4.22 (s, 1H), 3.87 (s, 3H), 3.78 (s, 3H), 3.37 (d, 1H, J = 11.4 Hz), 3.15 (t, 1H, J = 8.32, 14.5 Hz), 2.79 (t, 1H, J = 11.4, 15.6 Hz), 2.31 (d, 1H, J = 13.5 Hz), 2.21 (d, 1H, J = 13.5 Hz), 1.75 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.1, 167.9, 162.5, 149.5, 147.1, 136.0, 128.5, 128.1, 127.2, 111.2, 104,4, 71.2, 56.2, 52.1, 52.0, 45.3, 39.5, 27.2, 26.4, 25.2, 24.3, 21.0. (ESI) HRMS: m/z calcd for $C_{22}H_{24}N_4O_5Na$ 447.1644, found 447.1631 $[M]^{+\cdot}$.

5.1.9. (*S*)-3-Hydroxy-2-methoxy-7,8,9,10-tetrahydrobenzo[*e*]-pyrido[1,2-*a*][1,4]diazepine-6,12(5*H*,6a*H*)-dione 5a

To a stirred solution of **15a** (50 mg, 0.163 mmol) in CH_3CN (2 mL), were added NaI (70 mg, 0.460 mmol) and $Al(OTf)_3$ (13 mg, 20 mol %) at ambient temperature. The combined reaction mixture was stirred at the same temperature for 20 min, and then the reaction completion was monitored by the disappearance of starting material as indicated by TLC. Next, the solvent was removed under reduced pressure and the excess NaI was quenched with saturated sodium thiosulfate ($Na_2S_2O_3$). Next, the resulting product was extracted with ethyl acetate (3 × 20 mL), washed with $NaHCO_3$ solution (1 × 20 mL), brine (1 × 20 mL), and then dried over anhydrous Na_2SO_4. The final product was further purified by short length column chromatography through silica gel (100–200 mesh) by using ethyl acetate–hexane (90:10) as an eluent, to give compound **5a** in good yield (36 mg, 85%). Mp: 261–263 °C, $[\alpha]_D^{31} = +172$ (c 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.06 (br s, 1H), 7.36 (s, 1H), 6.51 (s, 1H), 6.34 (br s, 1H), 4.50 (d, 1H, J = 13.59 Hz), 4.13–4.16 (m, 1H), 3.93 (s, 3H), 2.97 (t, 1H, J = 9.82 Hz), 2.17–2.26 (m, 1H), 1.79–1.97 (m, 1H), 1.53–1.97 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.1, 155.2, 144.9, 134.9, 129.0, 128.2, 121.2, 116.1, 55.6, 42.3, 39.3, 36.1, 30.4, 29.2. (ESI) HRMS: m/z calcd for $C_{14}H_{16}N_2O_4Na$ 300.1193, found 300.1202 $[M]^{+\cdot}$.

5.1.10. (*S*)-3-(Benzyloxy)-2-methoxy-7,8,9,10-tetrahydrobenzo-[*e*]pyrido[1,2-*a*][1,4]diazepine-6,12(5*H*,6a*H*)-dione 5b

White solid (38 mg, 88%), mp: 195–197 °C, $[\alpha]_D^{32} = +248$ (c 1.0, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ 8.12 (br s, 1H), 7.31–7.41 (m, 6H), 6.42 (s, 1H), 5.15 (s, 2H), 4.49 (d, 1H, J = 13.91 Hz), 4.13–4.16 (m, 1H), 3.92 (s, 3H), 2.96 (t, 1H, J = 9.52 Hz), 2.17–2.23 (m, 1H), 1.89–1.95 (m, 1H), 1.54–1.73 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.6, 168.1, 151.1, 147.0, 135.8, 129.9, 128.6, 128.1, 127.1, 119.7, 112.6, 105.1, 70.8, 56.1, 51.2, 40.1, 23.1, 22.6, 19.0. (ESI) HRMS: m/z calcd for $C_{21}H_{22}N_2O_4Na$ 389.1477, found 389.1471 $[M]^{+\cdot}$.

Acknowledgments

This work has been supported by the Facility for Chemical Biology (Project SIP-0011, N. Shankaraiah) and FONDECYT (Project 1085308, L.S.S.).

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