Assembly of the TAN-1057 A/B Heterocycle from a Dehydroalanine Precursor

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Received 30 July 2000

Abstract: A tandem reaction process was used to form the tetrahydropyrimidinone core of the TAN-1057 A and B dipeptide antibiotics. Michael addition of ammonia to a dehydroalanine derivative, followed by intramolecular displacement of an *S*-methylisothiourea assembles the tetrahydropyrimidinone in one-step.

Key words: amino acids, antibiotics, cyclizations, nucleophilic additions, tandem reactions

Over the last decade, combinatorial chemistry[2] has developed into a powerful tool for the discovery of novel biologically active compounds. For some time, we have been interested in building combinatorial libraries around natural products, which are inherently disposed by evolutionary selection for the efficient display of chemical information in 3-dimensional space. Such projects, however, dictate different constraints from classical total synthesis, which is focused on reaching a single target molecule. The generation of libraries in a practical manner necessitates a sufficiently general and concise route, ideally employing building blocks that are readily available with high diversity. Peptides meet these criteria far more than any other natural products, but problems with bioavailability and stability severely limit their use as therapeutic agents. For these reasons, we have focused on alkaloid natural products that are derived from peptides but feature further transformations of the amide bonds or side-chains to give more drug-like heterocycles. Previously, we have applied this concept to tetramic acids,[3] fumitremorgins[4] and fumiquinazolines.[5]

Here, we report preliminary results[6] related to TAN-1057 (Figure), modified peptides recently isolated[7] by workers at Takeda from a culture of *Flexibacter* sp. PK-74 and PK-176, which display potent antibiotic activity against both Gram-positive and Gram-negative bacteria. Importantly, the compounds remain active against antibiotic-resistant strains, including methicillin-resistant *Staphylococcus aureus* (MRSA). In an in vivo model with MRSA infected mice, TAN-1057 A had an ED_{50} of 0.064 mg/kg, compared to 15.1 mg/kg for imipenem/cilastatin or 3.4 mg/kg for vancomycin (currently the clinical antibiotic of last resort).

An elegant total synthesis of the TAN-1057 antibiotics was achieved[8] by the Williams group, in which the tetrahydropyrimidinone heterocycle was assembled from chiral 2,3-diaminopropionic acid. The interconversion[7a] of TAN-1057 A and B under basic conditions suggested an alternative biomimetic disconnection to us, as the

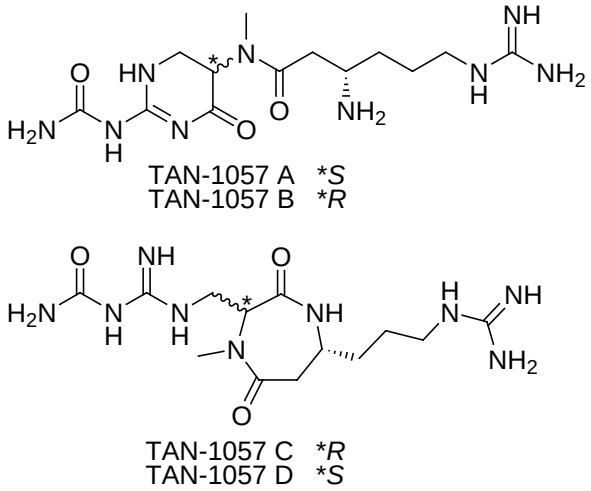
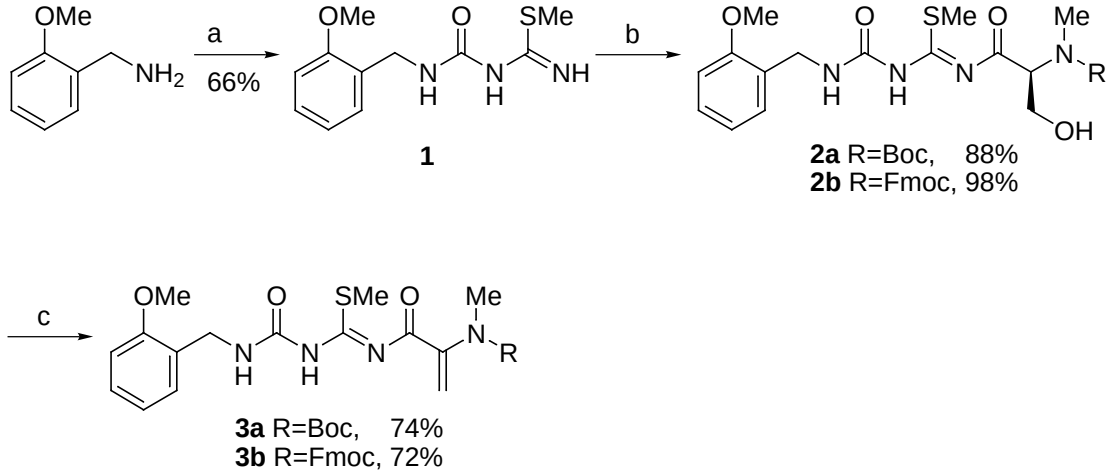


Figure Structures of TAN-1057 A–D

equilibration could be due to retro-Michael reaction and readdition. This implies that the heterocycle is biogenetically derived by conjugate addition of a nitrogen nucleophile to a dehydroalanine precursor, a reaction with synthetic precedent.[9] While our work was in progress, a second synthesis[10] of TAN-1057 A/B appeared in which the Michael addition of guanidine to dehydroalanine was attempted, but proved unsuccessful due to the formation of a 2:1 adduct by further reaction of the initial product.

Our approach was based on formation of the tetrahydropyrimidinone ring by a tandem reaction, in which Michael addition of ammonia to a dehydroalanine would be followed by the intramolecular displacement of an *S*-methylisothiourea. The isothiourea thus serves as a latent guanidine, and we had prior experience with such compounds in a combinatorial solid-phase synthesis[11] of unsymmetrical guanidines. A suitable model for our strategy was obtained by sequentially reacting *p*-nitrophenyl chloroformate with 2-methoxybenzylamine and the free base of *S*-methylisothiourea to afford **1** (Scheme 1), which was condensed with Boc and Fmoc protected *N*-methyl L-serine to give **2a** and **2b** respectively. Tosylation of the alcohol was accompanied by elimination to provide dehydroalanines **3a** and **3b**.

A number of protocols were examined for the key reaction of **3** with ammonia to yield tetrahydropyrimidinone **4** (Scheme 2). The best yield with **3a** (39%) was obtained using 1.5 molar equivalents of ammonia in methanol. Under these conditions, product **4a** precipitates from the re-



Reagents and conditions: (a) i. *p*-nitrophenyl chloroformate/(*i*-Pr)$_2$NEt/CH_2Cl_2, ii. 2-methyl-2-thiopseudourea/Et_3N; (b) *R*-*N*-methyl-L-serine/DCC/HOBt/CH_2Cl_2; (c) *p*-TsCl/Et_3N/DMAP/CH_2Cl_2

Scheme 1

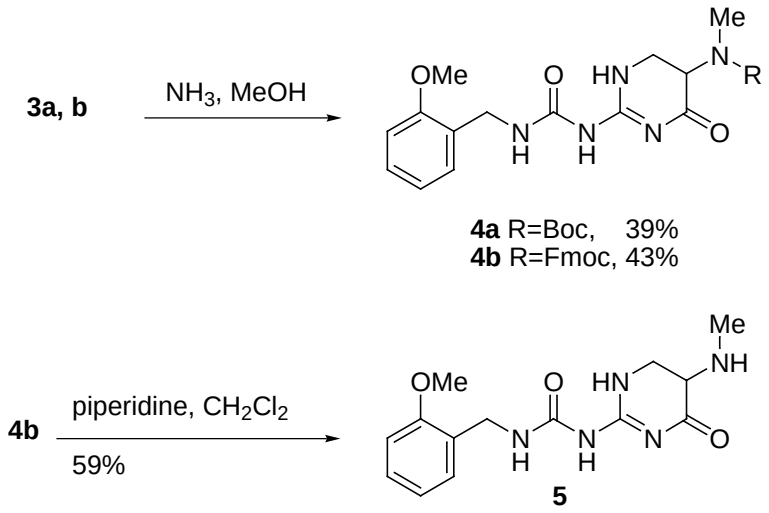
action mixture. Concentration of the filtrate gave a residue containing a mixture of byproducts including diamination adducts which could not be fully characterized. With the Fmoc protected dehydroalanine **3b**, larger amounts of ammonia were needed, and 4 molar equivalents yielded 43% of **4b**. Both **4a** and **4b** are suitable intermediates for further elaboration into TAN-1057 analogues. For example, deprotection of **4b** furnished **5**, and the secondary amine was then coupled with Fmoc-L-Ala-Cl to give the corresponding dipeptide as a mixture of diastereomers in 30% overall yield (unoptimized).

In summary, we have demonstrated the formation of the TAN-1057 A/B heterocycle by a tandem reaction initiated by Michael addition to a dehydroalanine, thus avoiding the use of more expensive unnatural amino acids. We are currently investigating additional variants of this process and adaptation of the conditions to solid-phase library synthesis, and further results will be reported in due course.

Melting points were taken on a hot stage apparatus and are uncorrected. ^1H and ^{13}C NMR were recorded in $CDCl_3$ at 400 MHz and 100MHz respectively. IR spectra were taken on KBr pellets. Low-resolution mass spectra were obtained by atmospheric pressure ionization, and high-resolution spectra (HRMS) by electrospray ionization.

2−Methoxybenzylcarbonyl-*S*-methyl-2-thiopseudobiuret (1)

To a cooled (ice-bath) solution of 2-methoxybenzylamine (129 μL, 1 mmol) in CH_2Cl_2 (25 mL) under N_2 was added pyridine (89 μL, 1.1 mmol) and *p*-nitrophenyl chloroformate (222 mg, 1.1 mmol). The mixture was stirred at r.t. until completion, as monitored by TLC (approximately 4 h). The solvent was then evaporated, the residue dried in vacuo and redissolved in anhyd acetone (30 mL), followed by the addition of *S*-methyl-2-thiopseudourea (99 mg, 1.1 mmol) and Et_3N (153 μL, 1.1 mmol). After stirring at r.t. for one



Scheme 2

day, the mixture was filtered, the filtrate concentrated, and chromatographed (eluting with hexanes and then 1:1 EtOAc/hexanes) to give 167 mg (66%) of **1** as a white solid; mp 93−95 °C.

^{1}H NMR: δ = 2.39 (s, 3 H), 3.86 (s, 3 H), 4.41 (d, 2 H, *J* = 6.1 Hz), 5.80 (br s, 1 H), 6.88 (d, 1 H, *J* = 8.2 Hz), 6.93 (t, 1 H, *J* = 7.4 Hz), 7.24−7.32 (m, 2 H).

^{13}C NMR: δ = 13.3, 39.6, 55.3, 110.2, 120.5, 126.9, 128.4, 129.2, 157.4, 163.0.

HRMS: *m*/*z* calcd for ($C_{11}H_{15}N_3O_2S$ + H) 254.0964, ($C_{11}H_{15}N_3O_2S$ + Na) 276.0783; found (M + H) 254.0963, (M + Na) 276.0783.

Anal. calcd for $C_{11}H_{15}N_3O_2S$: C, 52.15; H, 5.97; N, 16.59. Found: C, 52.19; H, 6.05; N, 16.16.

Isothiourea (2a)

To a mixture of *N*-*tert*-butoxycarbonyl-*N*-methyl-L-serine (339 mg, 1.55 mmol) and HOBt (199 mg, 1.48 mmol) in CH_2Cl_2 (50 mL) was added **1** (356 mg, 1.41 mmol) and DCC (105 mg, 1.48 mmol) at 0−5 °C. The resulting mixture was stirred for 20 h and filtered. The filtrate was concentrated, redissolved in a small amount of CH_2Cl_2, cooled with an ice-bath, and the white precipitate filtered off. The filtrate was washed (5% $NaHCO_3$) and the combined organic layers were collected, dried (Na_2SO_4), concentrated, and chromatographed (EtOAc/hexanes, 0:100 to 1:1) to give 562 mg (88%) of **2a** as a white foam; $[\alpha]_D^{25}$ +2.98 (*c* = 0.013, $CHCl_3$).

^{1}H NMR (mixture of rotamers): δ = 1.38 and 1.51 (1:1) (s, 9 H), 2.29 (s, 3 H), 2.93 and 3.02 (s, 3 H), 3.84−3.86 (m, 2 H), 3.85 (s, 3 H), 4.15−4.17 (m, 1 H), 4.40 (d, 2 H, *J* = 5.8 Hz), 6.00 (br s, 1 H), 6.88 (d, 1 H, *J* = 8.2 Hz), 6.92 (t, 1 H, *J* = 7.5 Hz), 7.25−7.29 (m, 2 H), 13.75 (br s, 1 H).

^{13}C NMR (mixture of rotamers): δ = 14.2, 28.1 and 28.2 (1:1), 33.1 and 34.3, 39.7, 55.3, 60.7, 61.7 and 63.3, 81.0 and 81.5, 110.2, 120.5, 126.0, 128.9, 129.6, 157.4, 161.4, 166.2, 170.1.

HRMS: *m*/*z* calcd for ($C_{20}H_{30}N_4O_6S$ + H) 455.1965, ($C_{20}H_{30}N_4O_6S$ + Na) 477.1784; found (M + H) 455.1964, (M + Na) 477.1784.

Anal. calcd for $C_{20}H_{30}N_4O_6S$: C, 52.85; H, 6.65; N, 12.33. Found: C, 52.39; H, 6.62; N, 12.06.

Isothiourea (2b)

To a mixture of Fmoc-*N*-methyl-L-serine (118 mg, 0.35 mmol) and HOBt (46.8 mg, 0.35 mmol) in CH_2Cl_2 (6 mL) were added **1** (81 mg, 0.32 mmol) and diisopropyl carbodiimide (54 μL, 0.35 mmol) at 0−5 °C. The resulting mixture was stirred overnight, filtered, and washed (5% aq $NaHCO_3$ and brine). The combined organic layers were dried (Na_2SO_4), concentrated, and chromatograped (EtOAc/hexanes, 0:100 to 3:2) to give 180 mg (98%) of **2b** as a white foam; $[\alpha]_D^{25}$ +16.6 (*c* = 0.005 M, $CHCl_3$).

IR (KBr): ν = 3422, 1701 cm^{-1}.

^{1}H NMR (mixture of rotamers): δ = 2.31(s, 3 H), 2.96 and 3.10 (s, 3 H), 3.65−3.97 (m, 2 H), 3.78 (s, 3 H), 4.21 (d, 2 H, *J* = 5.9 Hz), 4.30−4.36 (m, 2 H), 4.44−4.51 and 4.72 (m, 1 H), 4.58−4.62 (m, 1 H), 5.95 (br s, 1 H), 6.73 (t, 1 H, *J* = 7.4 Hz), 6.78−6.95 (m, 2 H), 7.16−7.49 (m, 5 H), 7.65−7.77 (m, 4 H), 13.73 and 13.96 (major) (s, 1 H).

^{13}C and Dept135: δ = 14.2 and 14.3 (SCH_3), 33.3 (NCH_3), 39.8 (NCH_2), 47.3 (Fmoc-CH), 55.2 (OCH_3), 60.4 and 60.7 (Ser-OCH_2), 62.5 (Ser-CH), 68.4 (Fmoc-CH_2O), 110.1, 119.9, 120.5, 125.3, 125.5, 127.1, 127.6, 128.8, 129.3, 141.2 (C_{quat}), 143.8 (C_{quat}), 144.3 (C_{quat}), 156.9 (C_{quat}), 157.3 (C_{quat}), 161.5 (C_{quat}), 166.4 (C_{quat}), 169.4 (C_{quat}).

MS: *m*/*z* (%) = 577 $[M + 1]^+$ (28), 414 (85), 324 (18), 180 (100), 146 (63).

Dehydroalanine (3a)

To a cooled solution (ice-bath) of **2a** (118 mg, 0.26 mmol) in CH_2Cl_2 (3 mL) was added anhyd Et_3N (108 μL, 0.78 mmol) and DMAP (3.2 mg, 0.026 mmol), followed by the dropwise addition of *p*-toluenesulfonyl chloride (62 mg, 0.31 mmol) in CH_2Cl_2 (1 mL). After 2 h the mixture was warmed to r.t. and stirred for 1.5 d. The solvent was then evaporated, and the residue chromatographed (EtOAc/hexanes, 1:3 to 3:7) to give 84 mg (74%) of **3a** as a white foam; mp 145−146 °C.

IR (KBr): ν = 3289, 1723, 1626 cm^{-1}.

^{1}H NMR: δ = 1.42 (s, 9 H), 2.34 (s, 3 H), 3.19 (s, 3 H), 3.86 (s, 3 H), 4.42 (d, 2 H, *J* = 6.1 Hz), 5.28 (s, 1 H), 5.69 (br s, 1 H), 6.07 (br s, 1 H), 6.87 (d, 1 H, *J* = 6.2 Hz), 6.92 (t, 1 H, *J* = 7.5 Hz), 7.25−7.34 (m, 2 H), 13.73 (br s, 1 H).

^{13}C NMR: δ = 14.3 (SCH_3), 28.0 (Boc- CH_3), 36.6 (NCH_3), 39.8 (NCH_2), 55.3 (OCH_3), 81.9 (Boc-C-O), 110.3, 112.0 (CH_2, alkene), 120.6, 126.1, 128.9, 129.5, 144.1 (C_{quat}, alkene), 157.5 (C_{quat}, guanidino), 161.6 (C_{quat}, ureido), 167.3 (C_{quat}, Boc-C=O and C_{quat}, Ser-C=O).

Anal. calcd for $C_{20}H_{28}N_4O_5S$: C, 55.03; H, 6.47; N, 12.83. Found: C, 55.12; H, 6.37; N, 12.87.

Dehydroalanine (3b)

To a cooled solution (ice-bath) of **2b** (241mg, 0.42mmol) in CH_2Cl_2 (12mL) was added Et_3N (145 μL, 1.05 mmol) and DMAP (5.1 mg, 0.042 mmol), followed by dropwise addition of *p*-toluenesulfonyl chloride (101 mg, 0.5 mmol) in CH_2Cl_2 (6 mL). After 2 h, the mixture was warmed to r.t. and stirred for 24 h. The solvent was evaporated, and the residue chromatographed (EtOAc/hexanes, 3:7 to 2:1) to give 168 mg (72%) of **3b** as a white foam.

^{1}H NMR (mixture of rotamers): δ = 2.27 and 2.30 (s, 3 H), 3.23 and 3.26 (s, 3 H), 3.83 and 3.83 (s, 3 H), 4.38 (br s, 5 H), 5.52 (br s, 1 H), 5.97−6.05 (br s, 2 H), 6.84−6.88 (m, 2 H), 7.23−7.36 (m, 6 H), 7.52 (m, 2 H), 7.72−7.74 (m, 2 H).

^{13}C NMR: δ = 14.3, 39.9, 47.0, 55.3, 68.4, 110.2, 119.8, 120.5, 125.1, 125.9, 127.0, 127.6, 128.9, 129.5, 141.2, 143.8, 157.4, 161.7, 162.1, 167.3.

MS: *m*/*z* (%) = 559 $[M + 1]^+$, (40), 396 (25), 179 (100).

HRMS: *m*/*z* calcd. for ($C_{30}H_{30}N_4O_5S$ + H) 559.2016, ($C_{30}H_{30}N_4O_5S$ + Na) 581.1835; found (M + H) 559.2015, (M + Na) 581.1834.

2-[*N*-(2'-Methoxybenzylcarbamoyl)amino]-5-(*N'*-methyl-*N'*-*tert*-butoxycarbonylamino)-1,4,5,6-tetrahydropyrimidin-4-one (4a)

To **3a** (196 mg, 0.45 mmol) in MeOH (10 mL) was added a solution of 2 M NH_3 in MeOH (344 μL, 0.69 mmol). The mixture turned cloudy after stirring for 8 h, and was kept for a further 12 h until complete consumption of the starting material. During this period, the amount of white precipitate increased gradually. The precipitate was collected and dried to obtain 71 mg (39%) of **4a** as a white solid; mp 198−199 °C.

IR 3449, 3229, 1699, 1608 cm^{-1}.

^{1}H NMR (mixture of rotamers): δ = 1.39 and 1.45 (s, 9 H), 2.75 and 2.80 (s, 3 H), 3.51−3.70 (m, 2 H), 3.83 (s, 3 H), 4.41 (d, 2 H, *J* = 5.6 Hz), 4.51 and 4.92 (br t, 1 H), 6.84 (d, 1 H, *J* = 8.2 Hz), 6.89 (t, 1 H, *J* = 7.4 Hz), 7.20−7.28 (m, 2 H), 9.62 (br s, 1 H), 10.34 (br s, 1 H).

^{13}C NMR (mixture of rotamers): δ = 28.3 (Boc), 32.0 and 32.5 (NCH_3), 38.7 (NCH_2), 39.5 and 39.9 (pyrimidinone-CH_2), 53.1 and 54.5 (pyrimidinone-CH), 55.2 (OCH_3), 80.3 and 80.7 (Boc-C-O), 110.1, 120.2, 126.5, 128.3, 128.5, 155.3 and 155.9 (C_{quat}, Boc-C=O), 156.6 and 157.2 (C_{quat}, guanidino), 160.1 (C_{quat}, ureido), 176.6 and 176.8 (C_{quat}, pyrimidinone-C=O).

Anal. calcd for $C_{19}H_{27}N_5O_5$: C, 56.28; H, 6.71; N, 17.27. Found: C, 56.55; H, 6.45; N, 17.23.

2-[*N*-(2'-Methoxybenzylcarbamoyl)amino]-5-(*N*'-methyl-*N*'-fluorenylmethylcarbonylamino)-1,4,5,6-tetrahydropyrimidin-4-one (4b)

Dehydroalanine **3b** (100 mg, 0.18 mmol) was dissolved in MeOH (3 mL) followed by the addition of 2 M NH_3 in MeOH (358 μL, 0.72 mmol). A white precipitate formed after stirring for 20 min. The reaction mixture was kept at r.t. for a further 20 h until the complete consumption of starting material and then filtered. The filtrate was evaporated off, the residue combined with the precipitate, and chromatographed to give 41 mg (43%) of **4b** as a white solid; mp 179−180 °C.

IR (KBr): ν = 3457, 3222, 1701, 1606 cm^{-1}.

^{1}H NMR (mixture of rotamers): δ = 2.80 and 2.84 (s, 3 H), 2.99 (m, 1 H), 3.64 (m, 1 H), 3.80 and 3.82 (s, 3 H), 3.95−4.45 (m, 3 H), 4.41 (d, 2 H, *J* = 6.6 Hz), 4.67 and 4.96 (m, 1 H), 6.83−6.94 (m, 2 H), 7.19−7.48 (m, 7 H), 7.59−7.68 (m, 2 H), 7.77 (d, 1 H, *J* = 7.6 Hz), 9.57 (br s, 1 H), 10.04 and 10.46 (br s, 1 H).

^{13}C NMR and Dept135 (mixture of rotamers): δ = 31.9 (NCH_3), 38.7 and 39.3 (Ser-CH_2), 47.1 (Fmoc-CH), 53.8 (Ser-CH), 55.3 (OCH_3), 66.5 (CH_2), 67.9 (CH_2), 110.2, 119.7, 119.9, 120.3, 124.3, 125.1, 126.4, 126.5, 127.0, 127.2, 127.6, 127.7, 128.4, 128.8, 141.2, 141.3, 143.7, 143.8, 155.9, 156.5, 156.7, 157.3, 159.6, 160.2, 175.7, 176.3.

MS: *m*/*z* (%) = 528 $[M + 1]^+$ (30), 365 (5), 179 (5), 64 (100).

HRMS: *m*/*z* calcd. for ($C_{29}H_{29}N_5O_5$ + H) 528.2248, ($C_{29}H_{29}N_5O_5$ + Na) 550.2067; found (M + H) 528.2247, (M + Na) 550.2044.

2-[*N*- (2'-Methoxybenzylcarbamoyl)amino]-5-*N*'-methyl-1,5,6-trihydropyrimidin-4-one (5)

Compound **4b** (20 mg, 0.038 mmol) was dissolved in 20% piperidine (1 mL) in anhyd CH_2Cl_2, and stirred at r.t. until the complete deprotection of Fmoc group as monitored by TLC (1.5 h). The resulting mixture was diluted with CH_2Cl_2, washed (H_2O, brine), and dried (Na_2SO_4). Filtration and concentration gave a residue, which was chromatographed to give 6.8 mg (59%) of **5** as a semi-solid.

^{1}H NMR: δ = 2.55 (s, 3 H), 3.15−3.19 (m, 2 H), 3.56−3.62 (m, 1 H), 3.73 (s, 3 H), 4.36 (d, 2 H, *J* = 5.7 Hz), 6.78 (d, 1 H, *J* = 8.12 Hz), 6.84 (t, 1 H, *J* = 7.36 Hz), 7.14 (d, 1 H, *J* = 7.2 Hz), 7.20 (t, 1 H, *J* = 9.71, Hz), 9.48 (br s, 1 H), 10.18 (br s, 1 H).

^{13}C NMR: δ = 35.0, 38.7, 42.4, 55.3, 56.5, 110.1, 120.3, 126.7, 128.2, 156.6, 157.1, 160.0, 179.5.

MS: *m*/*z* (%) = 306 $[M + 1]^+$ (100).

Acknowledgement

This work was supported by grants from the National Science and Technology Board of Singapore.

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Article Identifier:
1437-210X,E;2000,0,14,2127,2130,ftx,en;C03300SS.pdf