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Synthetic Studies Towards Bis-Heterodimeric Netropsin Analogs

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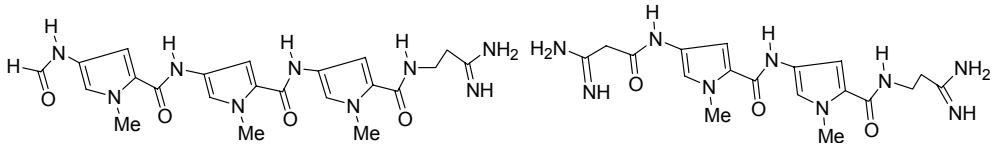
Abstract

An expedient synthetic method has been developed for the preparation of a properly functionalized pyrrole-imidazole template suitable for the construction of heterodimeric bis-lexitropsins. The pyrrole-imidazole carboxamide derivatives are connected through the nitrogen atoms of central heterocyclic rings by a tether of bis-ethoxyethane chain.

Keywords: Synthesis; Heterodimeric; Minor groove; Pyrrole; Imidazole.

Introduction

Distamycin (**1**)[1,2] and netropsin (**2**)[3,4] are crescent-shaped naturally occurring antiviral and antibiotic pyrrolecarboxamides. These oligopeptides have attracted researchers interest for their reversible non-covalent binding to the walls of the narrow minor groove of double-stranded DNA.[5,6] The binding occurs by means of hydrogen bonds between the NHs of the carboxamides and N^3 of adenine and O^2 of thymine, van der Waals contacts, and electrostatic interactions with a strong preference for sequences consisting of 4-5 AT base pairs.[7-9]



1 **2**

These ligands avoid binding to GC-containing sequences due to steric hindrance between the C-2 amino group of a guanine in GC-base pairs and a C-3 hydrogen atom of the pyrrole ring. Therefore, new sequence-selective analogs of netropsin and distamycin, named 'lexitropsins' or information reading molecules, have been

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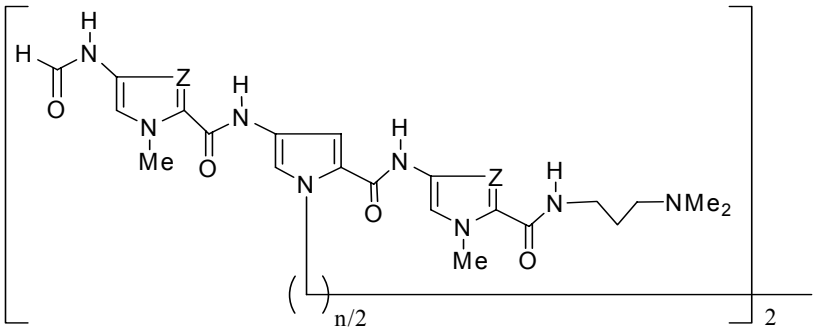
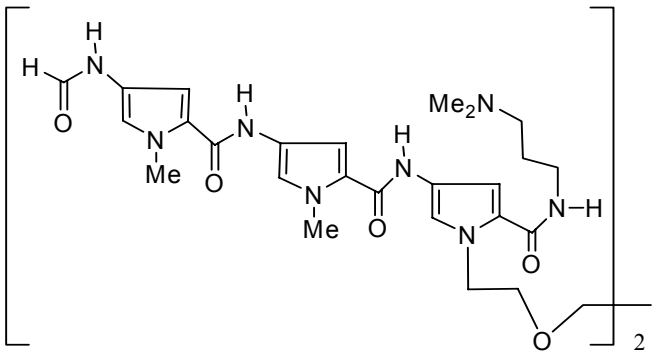
designed. These molecules yielded minor groove binding ligands with an increased tolerance for GC pairs by replacing a pyrrole with an imidazole moiety. This replacement transforms the steric interference into favored hydrogen bonding between the C-2 amino group of guanine in GC pair and a N-3 of the imidazole.[10-13] Increasing the number of heterocyclic units beyond five resulted in compounds with lower binding affinity to DNA, due to the incompatible phasing of the curvature which prevents maximum hydrogen bonding and van der Waals forces between the ligand and DNA.[14-16] NMR studies have shown that the binding of distamycin and netropsin occurs on AT or GC pairs along the floor of minor groove by a stoichiometry of 1:1 and in a 2:1 binding mode. The 2:1 binding mode is achieved by a widening of the minor groove of the DNA where the two drug molecules stacked side-by-side to opposite strands of DNA in an antiparallel fashion.[17-20] Therefore, two generations of linked bis-lexitropsin structures have been designed. In the first generation, named hairpin, the two units of oligopeptides, analogous to netropsin and distamycin, are covalently linked in a head-to-tail fashion by a linker.[21-23] In the second generation, named homodimeric cross-linked, the two units of distamycin analogs are linked through the central **3** or terminal pyrrole rings **4** *via* a polymethylene chain.[24-28] These covalently linked bis-lexitropsins were found to bind side-by-side in antiparallel fashion in the minor groove, recognized longer DNA sequences with stronger binding affinity compared to the monomer when the linker has the appropriate length.



3 (Z = N or CH; n = 5-10)



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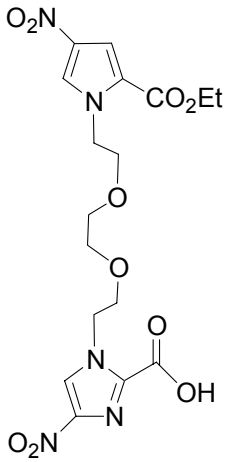

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In our effort to develop simple synthetic methods of biologically important compounds,[29] we wish to describe herein an efficient route for the formation of a

properly functionalized pyrrole-imidazole core unit **5**. This heterodimeric template is designed for the preparation of heterodimeric cross-linked bis-lexitropsins.

Experimental

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet 7199 FTIR spectrometer either in $CHC1_3$ or as KBr pellets. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer using $CDC1_3$ or DMSO-d_6 as solvent and internal standard. High-resolution mass spectra (HRMS) were obtained using a Kratos AEI MS-9 and MS-50 mass spectrometers.

*1-Methoxymethyl-4-nitro-2-trichloroacetylimidazole **(7)***

1-Methoxymethylimidazole (**6**)[30-31] (11.2 g, 0.1 mol) in dichlromethane (150 mL) was added to ice-child trichloroacetyl chloride (12.3 mL, 0.11 mol) in dichloromethane (250 mL) over 2 h. Stirring was continued for 2h, then triethylamine (17 mL), 0.12 mol) was added. The reaction mixture was stirred for 10 h and then concentrated to provide the crude product **6** (23.2 g, 90% yield) which was used in the next step without further purification.

*1-Methoxymethyl-4-nitro-2-trichloroacetylimidazole **(8)***

The crude product, 1-Methoxymethyl-2-trichloroacetylimidazole (**7**), (16 g, 62 mmol) was dissolved in dichloromethane (100mL) containing acetic anhydride (36 mL) and concentrated sulphuric acid (1 ml) in dichloromethane. After the mixture was cooled to –70 ^{0}C, fuming nitric acid (20 mL) was added dropwise over a period of 30 min. The temperature was raised to room temperature and stirring continued for 15 hours. The reaction mixture was then poured into iced water. The organic layer was separated and the aqueous layer was extracted with chloroform (200 mL). The combined organic layers was dried (Na_2SO_4) and concentrated to dryness under high vacuum to provide yellow oil **8**[30-31] (9.67 g, 51 % yield). IR ($CHCl_3$): 1774 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.52 (s, 3H, OCH_3), 5.80 (s, 2H, OCH_2), 8.19 (s, 1H, Im-H). ^{13}C NMR ($CDCl_3$): δ 52.4, 75.1, 106.5, 140.3, 142.3, 164.3, 178.2. HRMS: *m/z*, calcd. for $C_7H_6N_3O_4Cl_3$ 300.9424, found 300.9304 (M^+).

*1-Methoxymethyl-4-nitro-2-(2-trimethylsilylethyloxycarbonyl)imidazole **(9)***

To sodium hydride (1.68g, 48 mmol) in dry tetrahydrofuran (20 mL) was added dropwise 2-trimethylsilylethanol (5.5 ml, 38 mmol) in dry tetrahydrofuran (20 mL) with stirring over a period of 0.5 h. Then l-Methoxymethyl-4-nitro-2-trichloroacetylimidazole (**8**) (9.67 g, 32 mmol) in dry tetrahydrofuran (50 mL) was added dropwise over a period of 1 hour. Stirring was continued for an additional 15 hours before quenching the reaction with toluene-4-sulphonic acid (9.5 g, 50 mmol). The solvent was evaporated under vacuum and the residue was taken up with water (100 mL) and extracted with

ethyl acetate (4x100 mL). The combined organic layers was dried (Na_2SO_4) and concentrated to dryness. The resulting solid impregnated on silica was purified by flash chromatography using hexanes-acetone (3:1, v/v) as an eluent to provide pure compound **9** (5.25 g, 54.6% yield). M. P. 84-87 ^{0}C. IR (KBr): 1736 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.05 (s, 9H, $Si(CH_3)_3$), 1.18 (bs, 2H. CH_2Si), 3.43 (s. 3H, OCH_3), 4.44 (bs, 2H, OCH_2), 5.80 (s, 2H, OCH_2), 8.03 (s, lH, Im-H). HRMS: m/z, calcd. for $C_{11}H_{19}N_3O_5Si$ 301.1094, found 301.1108 (M^+). Anal. Calcd. for $C_{11}H_{19}N_3O_5Si$: (%) C, 43.80; H, 6.35; N, 13.94. Found (%) C, 43.58; H, 6.37; N, 14.10.

*1-H-4-nitro-2-(2-trimethylsilylethyloxycarbonyl)Imidazole **(10)***

To 1-methoxymethyl-4-nitro-2-(2-trimethylsilylethyloxycarbonyl)imidazole **9** (5.9 g, 19.58 mmol) in tetrahydrofuran (30 ml) was added 6N HCI (14 mL) and the mixture was stirred at room temperature for 7 days. The resulting precipitate is collected, washed with a small amount of water and dried over phosphorus pentoxide to provide pure deprotected imidazole **10** as a white solid (2.22 g, 44% yield). M. P. 228-230 ^{0}C. IR (KBr): 3218, 1731 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.06 (s, 9H, $Si(CH_3)_3$), 1.10 (m. 2H, CH_2Si), 4.40 (m, 2H, OCH_2), 8.50 (s, 1H, Im-H), 14.50 (exch bs, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 1.2, 16.3, 60.5, 132.7, 143.5, 153,9, 163.5. HRMS: m/z, calcd. for $C_9H_{15}N_3O_4Si$ 257.0832, found 257.0714 (M^+)*.* Anal. Calcd. for $C_{11}H_{19}N_3O_5Si$: (%) C, 42.01; H, 5.88; N, 16.30. Found (%) C, 42.60; H, 5.46; N, 15.54.

*1-(2-chloroethoxy)-2-N-[(4-nitro-2-ethyloxycarbonyl)pyrrole]ethoxyethane]eth-ane **(12)***

Sodium iodide (0.90 g, 30 mmol) and 1,8-bis(chloroethoxyethane) (3.74g. 20 mmol) in DMF (50 mL) were kept at 80 ^{o}C for 2 h. Then **11** (1.84 g, 10 mmol) and flame dried K_2CO_3 (2.76 g, 20 mmol) were added and the reaction mixture was stirred at the same temperature for 4 h. The solvent was evaporated under reduced pressure and the residue, taken up with water (250 mL), was extracted with ethyl acetate (3x100 mL). The combined organic layers were dried (Na_2SO_4), concentrated to dryness and the resulting crude product was purified by silica gel column chromatography using hexane-ethyl acetate (8:2) as an eluent to provide the desired product **12** (1.9 g. 44% yield). M. P. 180-182 ^{0}C. IR ($CHCl_3$): 1735 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.32 (t, J = 7 Hz, CH_3), 3.22 (t, J = 7Hz, 2H, CH_2I), 3.55 (s. 4H, $O(CH_2)_2O$), 3.68 (t, J = 7 Hz, 2H, CH_2O). 3.80 (t, J = 5 Hz, 2H, CH_2O), 4.38 (q, J = 7 Hz*,* 2H OCH_2), 4.56 (t, J = 5 Hz, 2H, OCH_2N), 7.44 and 7.80 (2d, J = 2 Hz*,* 1H each, 2 x PyH). ^{13}C NMR ($CDCl_3$): δ 8.5, 14.2, 44.6, 61.6, 72.2, 70.5, 71.4, 74.5, 113.4, 128.5, 129.8, 140.2, 158.8. HRMS: *mlz,* calcd. for $C_{13}H_{19}N_2O_6I$ 426.0288, found 426.0267 (M^+).

*1-[2-[N-(4-Nitro-2-(2-trimethylsilylethyloxycarbonyl))imidazole]ethoxyl-2-[2-[N-(4-nitro-2-ethyloxycarbonyl)pyrrole]ethoxy]ethane **(13)***

A flame-dried three-necked round bottom flask equipped with nitrogen inlet, an equalizer dropping funnel and a drying tube was charged with flame dried potassium carbonate (13.8 g, 100 mmol), imidazole compound **10** (3.1 g, 12 mmol) and pyrrole compound **12** (7.71 g, 18 mmol) in dry dimethylformamide *(*60 mL). The mixture was stirred for 7 days and then the solvent was evaporated under reduced pressure. The residue, taken up with water (250 ml), was extracted with ethyl acetate (3x100 ml). The combined organic layers were dried (Na_2SO_4), concentrated to dryness and the resulting crude product was purified by flash chromatography using hexane-ethyl acetate as an eluent to provide the desired product **13** (6.0 g, 90% yield). M. P. 149.151 ^{0}C. IR ($CHCl_3$): 1735, 1331 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 9H, $Si(CH_3)_3$), 1.20 (m, 2H, CH_2Si), 1.39 (t, J = 7Hz, 3H, CH_3), 3.54 (s, 4H, $O(CH_2)_2O$), 3.80 (q, J = 7 Hz, 4H, 2xCH_2N), 4.32 (q, 2H, OCH_2), 4.48 (m, 2H, OCH_2), 4.58 and 4.69 (3t. 3H each, OCH_2), 7.44 and 7.80 (2d, J + 2 Hz, 1H each, 2xPy-H), 8.04 (s, 1H, Im-H); HRMS: *m/z,* calcd. for $C_{22}H_{33}N_5O_{10}Si$ 555.1997, found 555.1876 (M^+). Anal. Calcd. for $C_{22}H_{33}N_5O_{10}Si$: (%) C, 47.56; H, 5.99**;** N, 12.60. Found (%) C, 46.85; H, 5.54; N, 12.85.

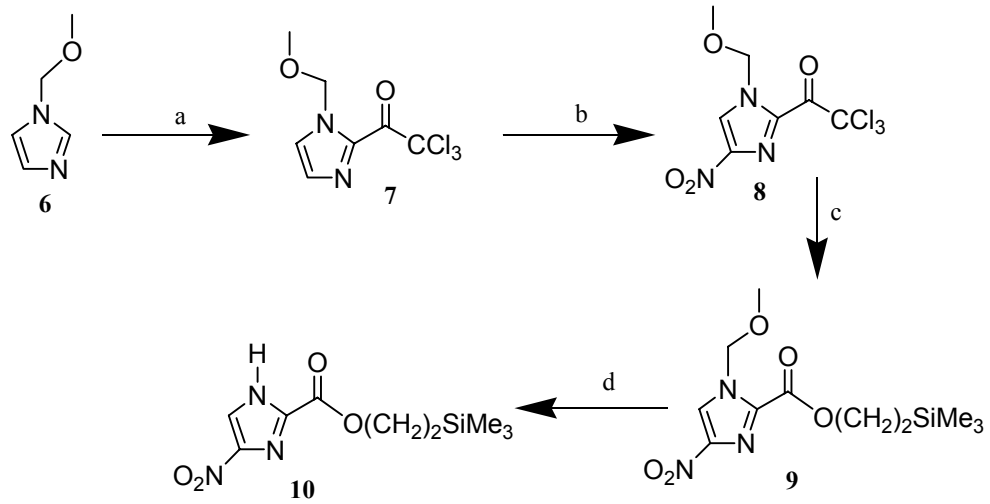
*1-[2-[N-(4-Nitro-2-(2-trimethylsilylethyloxycarbonyl))imidazole]ethoxyl-2-[2-[N-(4-nitro-2-carboxylic acid)pyrrole]ethoxy]ethane **(5)***

Hydrated tetrabutyl ammonium fluoride (2 mmol)) was added to **13** (0.55 g, 1mmol) dissolved in THF (30 mL). The reaction mixture was stirred for 6 h and then quenched with water (30 mL) and extracted with ethyl acetate (2x50 mL). The combined organic layers were dried (Na_2SO_4), concentrated to dryness and the resulting crude product was purified by flash chromatography using hexane-ethyl acetate (1:4) to provide the desired product **5** (0.38 g, 85% yield). M. P. 234-236 ^{0}C. IR (KBr): 3200-2880, 1739, 1728 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.29 (t, J = 7 Hz, 3H, CH_3), 3.55 (s, 4H, $O(CH_2)_2O$), 3.72-3.82 (m, 4H, 2xCH_2O), 4.32 (q, J = 7 Hz, 2H, OCH_2), 4.43, 4.62 (t, J = 7 Hz, 2H each, NCH_2), 7.46 and 7.77 (2d, 1H each, J = 2 Hz, 2xPy-H), 8.14 (s, 1H, Im-H). ^{13}C NMR ($CDCl_3$): δ 14.4, 43.8, 44.6, 58.9, 68.7, 70.1, 70.3, 113.3, 128.6, 128.6, 138.6, 138.9, 141.6, 155.7, 162.4, 173.4. HRMS: *m/z,* calcd. for $C_{17}H_{21}N_5O_{10}$ 455.1288, found 455.1092 (M^+). Anal. Calcd. for $C_{17}H_{21}N_3O_{10}$ (%) C, 44.84; H, 4.65**;** N, 15.38. Found (%) C, 45.20; H, 4.78; N, 14.85.

Results and Discussion

The synthesis of the heterodymeric core unit **5** commenced with specially functionalized imidazole and pyrrole units as shown in Scheme 1. Thus, 1-methoxymethylimidazole (**6**) was smoothly acylated with trichloroacetyl chloride in dichloromethane to afford crude 1-methoxymethyl-2-trichloroacetylimidazole (**7**). The crude product was used in the next step without purification. Therefore, nitration of **7**

with nitronium acetate generated from fuming HNO_3 and acetic anhydride in the presence of catalytic amount of H_2SO_4 in dichloromethane furnished **8** in low yield (25%). Improving the yield to 51 % was achieved by dropwise addition of fuming nitric acid to a solution of **7** in dichloromethane containing acetic anhydride and a catalytic amount of concentrated sulfuric acid at low temperature (-70^{o}C). After warming to room temperature the reaction mixture was stirred for additionally 15 h. Neither additional portions of the nitrating agent nor prolonged reaction time improved the yield.



Scheme 1

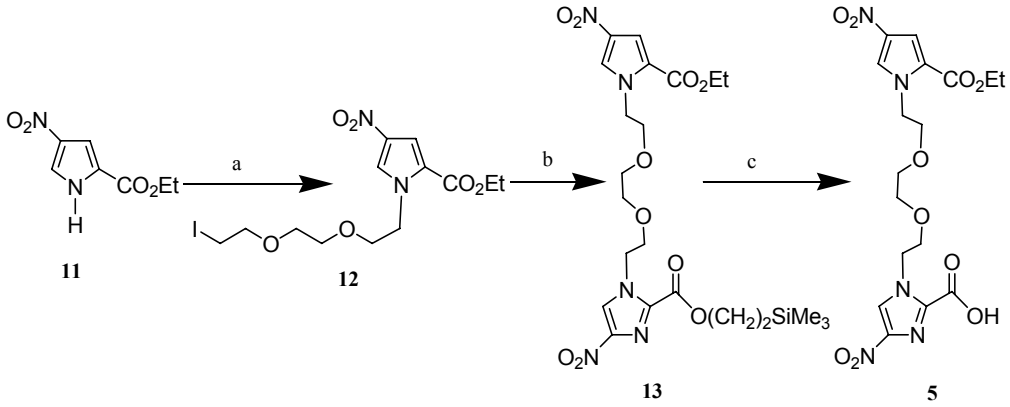
a) Cl_3CCOCl, CH_2Cl_2, 90%. b) HNO_3, Ac_2O, $H_2SO_{4(cat)}$, CH_2Cl_2, 51%. c) NaH, $HO(CH_2)_2SiMe_3$, THF, 55%. d) 6N HCl, THF, H_2O, 44%.

With the nitrated imidazole derivative **8** in hand, we turned to the process of converting the trichloroacetyl to an ester group that can be transformed to the corresponding acid under specific conditions. Accordingly, **8** was treated with trimethylsilylethoxide in dry THF to furnish the expected product **9** in a reasonable yield (55%). The ^{1}H NMR spectrum for compound **9** shows a singlet at δ 0.05 characterestic for –$SiMe_3$ and a broad singlet at δ 1.18 for the methylene protons next to silicon. Furthermore, its HRMS showed the molecular ion at *m/z* 300.1092 (M^+) in agreement with the molecular formula $C_{11}H_{19}N_3O_5Si$.

Cleavage of N-methoxymethyl protecting group in substrate **9** was achieved by stirred with 6N HCl in THF to afford the desired product **10** as a solid that precipitated in the reaction mixture and isolated in pure form by vacuum filtration. It is noteworthy that conducting the reaction at higher temperature (64 ^{0}C) or in concentrated HCl at room temperature resulted in a dark-colored solution containing only traces of **10** as indicated by TLC analysis.

With convenient access to **10**, we then focused on the preparation of the properly functionalized pyrrole unit. Therefore, coupling of the easily accessible ethyl 4-nitropyrrol-2-carboxylate (**11**) and 1,8-bis(choroethoxyethane) to form the N-alkylated pyrrole unit **12** employing the conditions shown in Scheme 2 that we have previously established with several 1,n-dihaloalkanes.[24,25,29] The product of the coupling process was isolated from the reaction mixture by silica gel column chromatography of the residue obtained after concentrating the organic extracts. The ^{1}H NMR spectrum of **12** displayed the methylene units at *δ* 3.22, 3.55, 3.68, 3.80, 4.38 and 4.56. Furthermore, its HRMS shows the molecular ion at *m/z* 426.0266 (M^+) which is in good agreement with the molecular formula $C_{13}H_{19}N_2O_6I$.

Having completed the assembly of the imidazole and pyrrole subunits, it remained to combine these two units. Thus, the imidazole **10** and the pyrrole **12** were stirred for seven days in dry DMF in the presence of excess flame-dried K_2CO_3. The heterodimeric compound **13** was purified on a silica gel column eluting with ethyl acetate-hexane (1:2). It was fully characterized using HRMS and NMR. Its HRMS showed the molecular ion at *m/z* 555.1876 (M^+), in agreement with the molecular formula $C_{22}H_{33}N_5O_{10}Si$. The ^{1}H NMR spectrum of **13** provided conclusive evidence for its formation.



Scheme 2

a) K_2CO_3, 1,8-bis(chloroethoxyethane), KI, DMF, 44%. b) K_2CO_3, DMF, 90%. c) Bu_4NF, THF, H_2O, 85%.

Having completed the assembly of the imidazole and pyrrole subunits, it remained to combine these two units. Thus, the imidazole **10** and the pyrrole **12** were stirred for seven days in dry DMF in the presence of excess flame-dried K_2CO_3. The heterodimeric compound **13** was purified on a silica gel column eluting with ethyl acetate-hexane (1:2). It was fully characterized using HRMS and NMR. Its HRMS showed the molecular ion at *m/z* 555.1876 (M^+), in agreement with the molecular formula $C_{22}H_{33}N_5O_{10}Si$. The ^{1}H NMR spectrum of **13** provided conclusive evidence for its formation.

Finally, selective hydrolysis of compound **13** was achieved in aqueous THF solution containing excess Bu_4NF to afford the required template **5** in excellent yield (92%) as a pale yellow solid. The final product **5** was fully characterized using HRMS and ^1H NMR. The HRMS of **5** showed the molecular ion at *m/z* 455.1092 (M^+), corresponding to the molecular formula $C_{17}H_{21}N_5O_{10}$. The ^1H and ^{13}C NMR spectra of **5** were also in agreement with the proposed structure.

Conclusion

We have presented here, for the first time, a synthetic protocol for preparing properly functionalized heterodymeric template suitable for the synthesis of wide range of lexitropsins. This template possesses a free carboxylic acid suitable for direct amide formation and an ester group amendable for further chemical transformation. The starting materials, used in this strategy, are readily accessible. Moreover, all the reactions were conducted in multi-grams scale.

Acknowledgments

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