Pergamon

Tetrahedron Letters, Vol. 35, No. 41, pp. 7577-7580, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01628-3

Synthesis of Novel Cross-Linked bis-Lexitropsins

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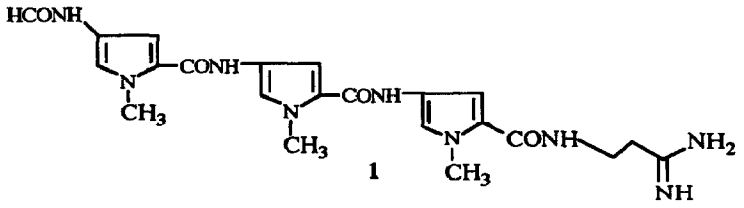
Abstract: *The synthesis of covalently cross-linked bis-lexitropsins **14**, **15** and **16** designed to bind to specific nucleotide sequences in the minor groove of double-helical DNA is described.*

Lexitropsins, a class of antitumor antibiotic oligopeptides structurally related to distamycin **1**, bind reversibly to specific nucleotide sequences in the minor groove of double-helical B-DNA blocking its template function.[1] The natural antibiotic distamycin recognizes $(A/T)_5$ sequences.[2] Rational structural modification led to the design of ligands with an increased tolerance for the GC base pair and ultimately the capacity to recognize mixed sequences. Replacing a pyrrole ring of distamycin with an imidazole ring, for example, allows formation of hydrogen bonds between G(2)-NH_2 in the minor groove and the N3 of the imidazole moiety.[1] Recently NMR studies have indicated that the minor groove can accommodate two peptidic lexitropsins in an antiparallel side by side manner.[3] These findings have prompted the design and synthesis of cross-linked lexitropsins, wherein the two peptides are connected through the nitrogens of the central pyrrole rings via a polymethylene chain.[4] This new generation of covalently cross-linked bis-lexitropsins show stronger binding when the linker is the appropriate length



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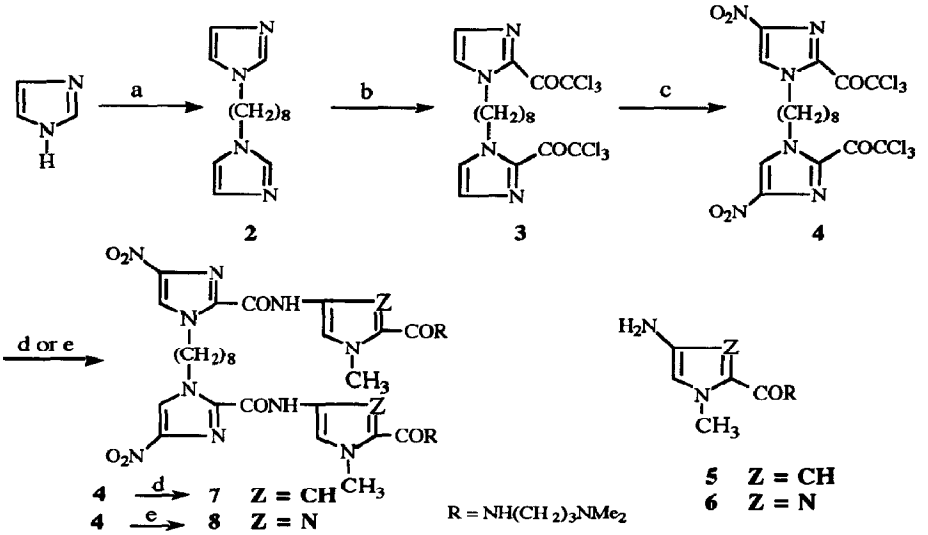
with double-helical DNA[4c] and higher specificity compared with the monomer.[1b,c] Herein we report a convenient method for the preparation of a new generation of such covalently cross-linked bis-lexitropsins, in which the central pyrrole ring is replaced with an imidazole ring, starting from the corresponding imidazole.

Our synthesis of cross-linked bis-lexitropsins began with the preparation of the properly functionalized central bis-imidazole unit in which the linker arm was introduced at an early stage. Alkylation of imidazole with 1,8-dibromooctane was successfully achieved by dropwise addition of the alkyl halide to the potassium salt of imidazole, generated in situ from potassium and imidazole in refluxing tetrahydrofuran furnished the corresponding di-*N*,*N*'-imidazolyloctane **2** in 82% yield. The addition of 1,8-dibromooctane to the reaction mixture just after the addition of the potassium metal to the

solution of imidazole helped to shorten the time necessary for the metal to dissolve completely from several hours to one. This avoided the use of a large volume of the solvent required to allow efficient stirring of the suspension. The methodology used for the introduction of the key trichloroacetyl functionalities to di-*N*,*N*'-imidazolyloctane was analogous to that of the corresponding *N*-methylimidazole.[5] Thus, when the reaction was performed it gave the expected product **3** in 80% yield.

With convenient access to **3** secure, we then focused on the completion of the synthesis of the properly functionalized central bis-imidazole unit. Toward this end, the nitration of **3** was investigated. We found, after several attempts, that the desired dinitro derivative **4** could be isolated in moderate yield (48%) upon exposure of **3** to excess nitronium acetate.[6]

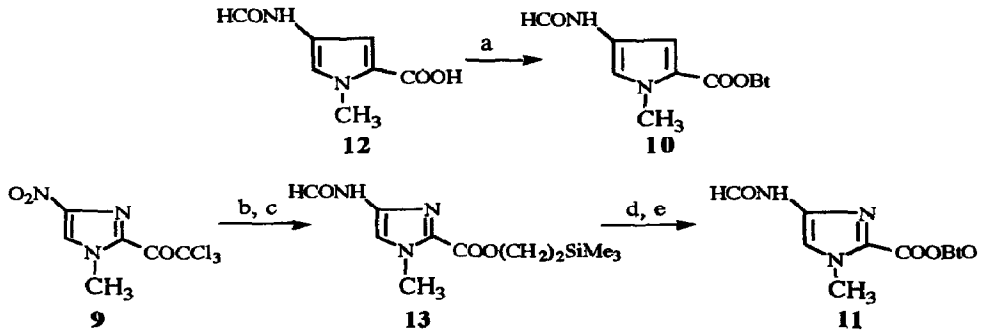


a) K, THF, then $Br(CH_2)_8Br$; b) CCl_3COCl, CH_2Cl_2, Et_3N; c) Ac_2O, HNO_3, H_2SO_4; d) **5**, DMF, 50°C, Et_3N; e) **6**, DMF, 50°C, Et_3N.

We next proceeded to examine the coupling of dinitro derivative **4** with the unstable amines **5** and **6**. The former amine was prepared following the the procedure of Shibuya[5] and the latter was synthesized from the known 4-nitro-2-trichloroacetyl-1-methylimidazole **9**[5] by condensation with 3-dimethylaminopropylamine in THF folowed by hydrogenation over PtO_2. Condensation of **4** with the unstable amine **5** in the presence of base (Et_3N) at 50°C in DMF gave the corresponding amide **7** in 66% yield. Under similar conditions condensation of **4** with the unstable amine **6** yielded the tetra-imidazole derivative **8** in 76% yield.

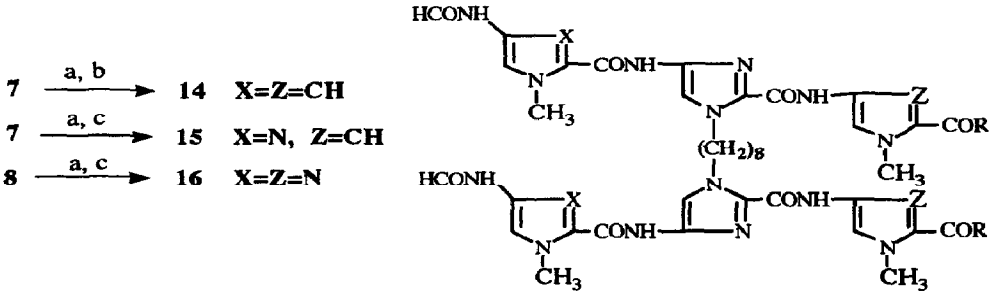
Having completed the assembly of the common four ring core intermediates **7** and **8**, it remained to introduce the heterocyclic moieties (imidazole and pyrrole) carrying the formyl group. The methodology chosen for this synthesis is designed to minimize the number of reaction steps required to convert amides **7** and **8** to the final products due to the high polarity of these intermediate which caused difficulties in purification. Following the success of our recent synthesis of hexapyrrole cross-linked lexitropsins, we sought to apply similar methodology to the present problem.[4c] Thus, the activated esters

10 and **11** were prepared. The former activated ester was easily obtained as a pure solid by treatment of the corresponding acid **12**[7] with *N*-hydroxybenzotriazole hydrate (HOBt) and 1-(-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in DMF. The latter activated ester was prepared from **9**[5] in four steps. First, compond **9** was submitted to condensation with lithium 2-trimethylsilylethoxide, followed by hydrogenation and treatment with excess acetic formic anhydride to provide **13**. This compound on exposure to tetrabutylammonium fluoride followed by treatment with 3-hydroxy-1,2,3-benzotriazin-4(*3H*)-one (HOBtO) and EDCI in DMF, gave the activated ester **11** as a pure solid.



a) HOBt, EDCI, DMF; b) $HOCH_2CH_2SiMe_3$, BuLi, THF, -10°C, then **9**; c) i) H_2, Pd(C), MeOH; ii) $CH_3COOCHO$; d) Bu_4NF, THF; e) HOBtO, EDCI, DMF.

With activated esters **10** and **11** in hand, we then conducted the last step towards the synthesis of the target lexitropsins **14**, **15** and **16**. Catalytic hydrogenation with PtO_2 in methanol of the dinitro intermediates **7** and **8** resulted in the formation of the corresponding unstable diamino derivatives which, after the removal of the solvent, were applied to the final step without purification. Treatment of the unstable diamino derivative of **7** with the activated ester **10** in DMF at 50°C in the presence of trimethylamine afforded the expected lexitropsin **14**[8] in 28% yield after flash chromatography ($MeOH:CHCl_3:NH_4OH$=47:47:6) and preparative TLC purification. In a similar manner, the unstable diamino derivatives of **7** and **8** were coupled with the activated ester **11** to give the cross-linked lexitropsins **15**[9] and **16** in 38 and 36% yield respectively.



a) H_2/Pt, MeOH; b) **10**, DMF, Et_3N, 50°C; c) **11**, DMF, Et_3N, 50°C.

The successful approach described herein enabled us to synthesize a number of covalently cross-linked bis-lexitropsins with different heterocyclic moieties at both ends. The binding of these lexitropsins to the minor groove of the double-helical DNA and their sequence preferences will be reported in a future publication.

Acknowledgment We thank the Natural Sciences and Engineering Research Council of Canada for financial support (to J. W. Lown) to this research program.

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6. *Synthesis of 4 from 3:* Compound **3** (4.03 g, 7.5 mmol) was added to nitronium acetate generated by adding fuming HNO_3 (4 mL) dropwise to acetic anhydride (30 mL) and conc. H_2SO_4 (0.1 mL) at -10°C. The reaction mixture was stirred at ambient temperature for 18 h and then warmed slowly to 70°C for 2 h. It was then cooled to 0°C and HNO_3 (2 mL) added dropwise. The reaction mixture was warmed to 70°C again for 2 h before ethyl acetate was added. The organic layer was washed with water, dried and concentrated. The residual solid was chromatographed on silica gel (CH_2Cl_2) to give dinitro derivative **4** (2.4 g, 48%).
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8. For **14**: ^{1}H NMR (DMSO-d_6/Acetone-d_6): 10.18, 9.98, 9.78 (each s, 2H each, N**H**), 8.15 (bs, 2H, **H**CO), 8.08 (t, J = 5 Hz, 2H, N**H**), 7.58 (s, 2H, imidazole-**H**), 7.32, 7.26, 7.08 and 6.94 (each d, J = 2 Hz, 2H each, pyrrole-**H**), 4.49 (t, J = 7 Hz, 4H, NC**H**$_2$), 3.90 and 3.85 (each s, 6H each, NC**H**$_3$), 3.30 (4H, obscured by the H_2O signal), 2.34 (t, J = 7 Hz, 4H, C**H**$_2$), 2.20 (s, 12H, N(C**H**$_3$)$_2$), 1.82 (m, 4H, C**H**$_2$), 1.66 (p, J = 7 Hz, C**H**$_2$), 1.33 (bs, 8H, C**H**$_2$).
9. For **15**: ^{1}H NMR (DMSO-d_6): 10.55, 10.38, 9.46 (each s, 2H each, N**H**), 8.23 (d, J = 2 Hz, 2H, **H**CO), 8.13 (t, J = 6 Hz, 4H, N**H**), 7.57 and 7.56 (each s, 2H each, imidazole-**H**), 7.20, and 6.95 (each d, J = 2 Hz, 2H each, pyrrole-**H**), 4.43 (t, J = 7 Hz, 4H, NC**H**$_2$), 4.00 and 3.81 (each s, 6H each, NC**H**$_3$), 3.15 (q, J = 6 Hz, 4H, C**H**$_2$), 2.23 (t, J = 7 Hz, 4H, C**H**$_2$), 2.13 (s, 12H, N(C**H**$_3$)$_2$), 1.74 (m, 4H, C**H**$_2$), 1.58 (p, J = 7 Hz, 4H, C**H**$_2$), 1.27 (bs, 8H, C**H**$_2$).

(*Received in USA* 27 *July* 1994; *revised* 8 *August* 1994; *accepted* 23 *August* 1994)