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Novel distamycin analogues: facile synthesis of cholesterol conjugates of distamycin-like oligopeptides

Santanu Bhattacharya* and Mini Thomas

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India

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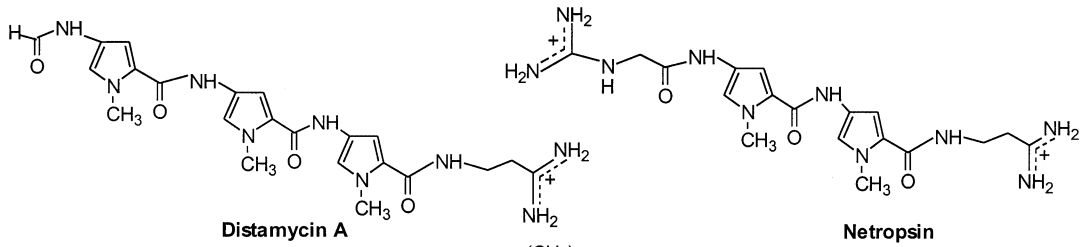
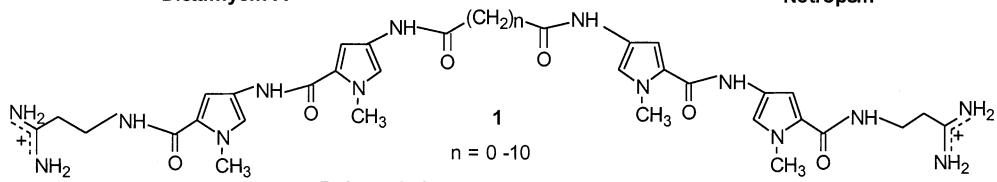
Abstract—A facile route towards the synthesis of the first examples of cholesterol-conjugated distamycin analogues is described. These compounds retain their strong binding capacity to double-stranded (ds)-DNA. Aqueous suspensions of these analogues exhibited closed, vesicle-like structures under transmission electron microscope. © 2001 Published by Elsevier Science Ltd.

The development of protocols to design low molecular mass systems that can manipulate protein–DNA or protein–protein interactions is a high priority initiative for chemical biologists interested in achieving gene regulation.[1] In this context, molecular design of analogues of naturally occuring antiviral compounds such as Distamycin A (Dst) and Netropsin (Nt), which bind at the minor groove of ds-DNA, represents an effective strategy for the development of antineoplastic agents.[2] For instance, Lown et al. reported the synthesis of several bis-netropsin analogues, such as **1**, obtained upon covalent connection of two symmetrical Nt fragments through a polymethylene or related lipophilic dicarboxylic acid.[3] Many of these compounds showed a substantial increase in potency in tumor cell cytotoxicity, and were also found to be more selective toward human tumor cells compared to their parent compounds. Additionally, these molecules also exhibited significant activity against retroviruses including HIV-1.[3]

Cytofectins that bear DNA binding polyamines, such as spermidine attached to cholesterol, have been reported to show promise as efficient DNA transfection agents.[4] It occurred to us that cholesterol-tethered distamycins should therefore be tested for their gene transfection abilities. Moreover, DNA is known to undergo structural transformation on binding to cationic lipids,[4,5] whereas minor groove binders are known to stabilize the *B*-form of ds-DNA.[6] Minor groove binding cationic cholesterol conjugates would therefore be useful for the evaluation of the relative importance of these factors on the ds-DNA complexation properties. Additionally, linking of Dst based oligopeptides to





Polymethylene linked netropsin dimers

* Corresponding author. Swarnajayanti Fellow, Department of Science & Technology. E-mail: sb@orgchem.iisc.ernet.in

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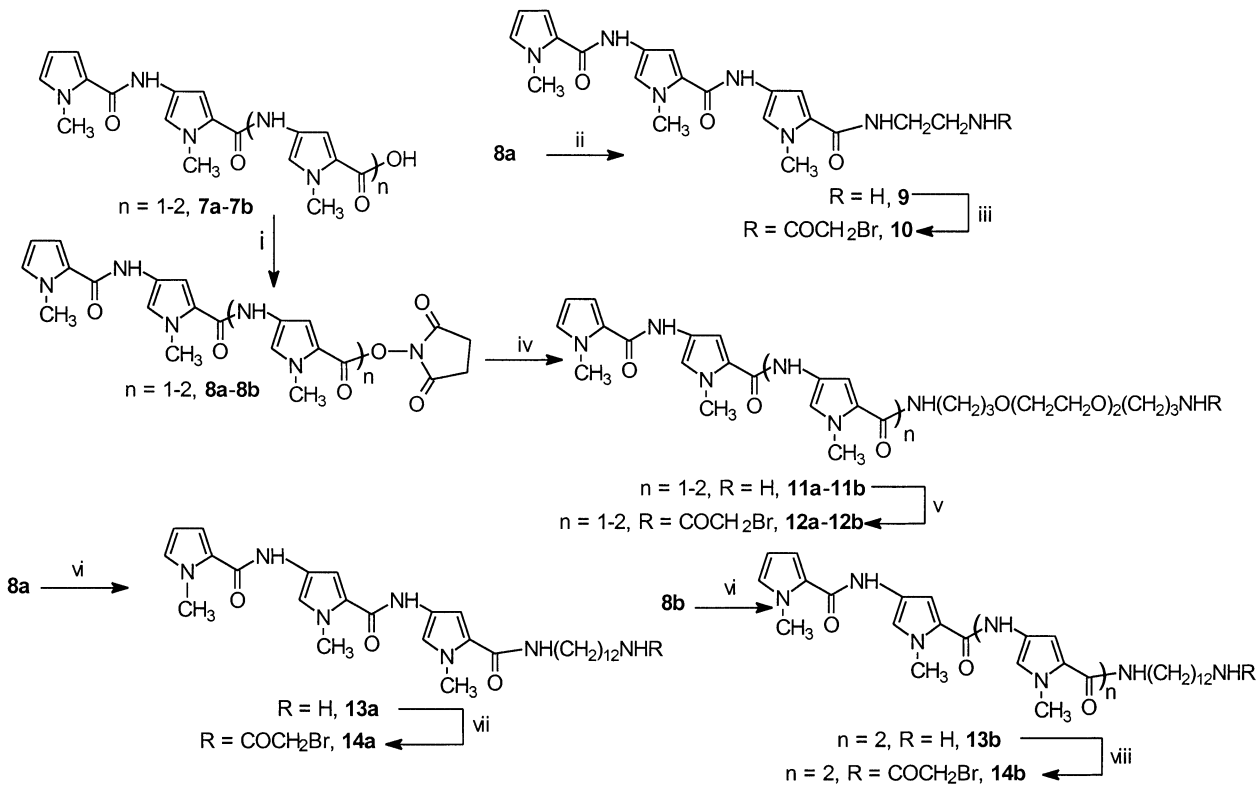
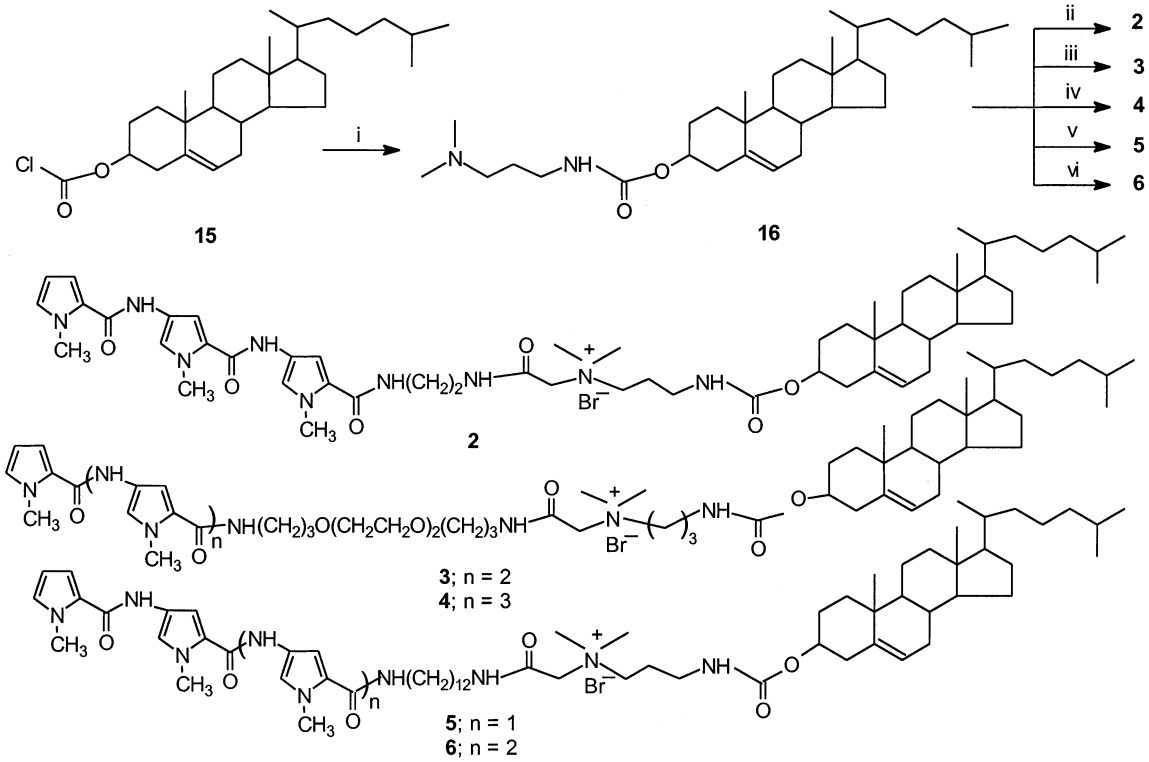
steroid units such as cholesterol might also improve the resulting compounds' partitioning into the cell membrane, and hence should improve their cellular uptake.

The synthesis of these novel Dst derivatives began with the preparation of the carboxylic acids **7a** and **7b** starting from methyl-4-nitro-*N*-methylpyrrole-2-carboxylate by the successive reduction and coupling of 4-nitro-*N*-methylpyrrole-2-carbonyl chloride to the growing peptide chain, and terminating the chain growth with *N*-methylpyrrole-2-carbonyl chloride, followed by the hydrolysis of the methyl esters.[7] Compounds **7a** and **7b** were then converted to the corresponding succinimide esters employing HOSU in the presence of DCC in dry DMF, as shown in Scheme 1. Most of the DCU formed during the reaction was removed by filtration after the addition of EtOAc. DMF was removed from this by aqueous work up and the solid obtained upon evaporation of the EtOAc layer was purified by column chromatography employing 4% MeOH in $CHCl_3$ to obtain either **8a** or **8b**, both in 80% yield. Compound **8a** or **8b** was dissolved in dry $CHCl_3$ and was then added dropwise to a separate solution of the selected diamine (2 equiv.) in dry $CHCl_3$. The resulting solutions were allowed to stir at rt until TLC indicated complete disappearance of the starting materials (**8a** and **8b**) in each case. Solvent evaporation followed by column chromatography on silica gel using first 4% MeOH in $CHCl_3$, followed by 18:78:2 MeOH/$CHCl_3$/aq. NH_3 afforded the desired products **9**, **11a–b** and **13a–b** in 74, 65, 65, 70 and 72% yields, respectively. Facile bromoacetylation of the individual monoamines **9**, **11a–b** or **13a** could be accomplished in dry $CHCl_3$ at 0°C in the presence of Et_3N. Solvent removal followed by gravity driven column chromatography on silica gel using 1–4% MeOH in $CHCl_3$ as eluent afforded the corresponding bromoacetylated amides **10**, **12a**, **12b** and **14a** in 74, 70, 72 and 65% isolated yields, respectively. Since **13b** was insoluble in organic solvents commonly used for acylation reactions, an alternative strategy was used for achieving bromoacetylation. Accordingly **13b** was first dissolved in 1:2 MeOH/$CHCl_3$ and the HOSU ester of bromoacetic acid was added to the above solution. Upon stirring, this reaction was complete in approximately 2 h and the purified product, **14b**, was obtained in 70% yield upon column chromatography on silica gel (1–4% MeOH in $CHCl_3$).

In a separate set up, cholesteryl chloroformate **15** was reacted with *N*,*N*-dimethyl-1,3-diamino-propane (Scheme 2). The product, **16**, was isolated in 90% yield upon column chromatography on neutral alumina (2–6% MeOH in $CHCl_3$). This was reacted with either of the bromides **10**, **12a–b**, **14a–b** in a screw-capped pressure tube in dry $CHCl_3$ or $CHCl_3$/MeOH to obtain the final products **2–6** in 92, 92, 90, 70 and 72% yields, respectively, upon column chromatography (silica gel, 4–10% MeOH in $CHCl_3$). While in the case of **3** and **4**, the quarternization reaction went to completion within



Scheme 1. *Reagents, conditions and yields*: (i) HOSU, DCC, dry DMF, 4 h (80% each); (ii) $NH_2(CH_2)_2NH_2$, dry $CHCl_3$, rt, 1 h (74%); (iii) $BrCOCH_2Br$, Et_3N, dry $CHCl_3$, −5°C, 20 min (70%); (iv) 4,7,10-trioxa-1,13-tridecanediamine, dry $CHCl_3$, rt, 1 h (65% each); (v) $BrCOCH_2Br$, Et_3N, dry $CHCl_3$, −5°C, 20 min (**12a**, 70%; **12b**, 72%); (vi) $NH_2(CH_2)_{12}NH_2$, dry $CHCl_3$, rt, 1 h (**13a**, 70%; **13b** 72%); (vii) $BrCOCH_2Br$, Et_3N, dry $CHCl_3$, −5°C, 20 min (65%); (viii) $BrCH_2COON(COCH_2)_2$, dry $CHCl_3$, rt, 2 h (70%).



Scheme 2. *Reagents conditions and yields*: (i) *N*,*N*-dimethyl-1,3-diaminopropane, dry $CHCl_3$, 0°C, 10 min, then rt, 30 min (90%); (ii) **10**, dry $CHCl_3$, pressure tube, 100°C 12 h (92%); (iii) **12a**, dry $CHCl_3$, sealed tube, 70°C, 2 h (92%); (iv) **12b**, dry $CHCl_3$, sealed tube, 70°C, 2 h (90%); (v) **14a**, dry $CHCl_3$/MeOH (1:2), sealed tube, 24 h, 100°C (70%); (vi) **14b**, dry $CHCl_3$/MeOH (1:2), sealed tube 24 h (72%).

2 h, it took about 12 h for **2** and as long as 24 h in the case of **5** and **6**. All the numbered intermediates and the final compounds were characterized by IR, ^{1}H NMR and mass spectral analysis (ESI-MS or MALDI-TOF), which were consistent with their given structures.[8]

Stable aqueous suspensions of the newly synthesized cytofectins could be obtained by an ethanol injection protocol.[9] Examination under transmission electron microscopy revealed the presence of vesicle-like organizations in these suspensions. In addition, these suspensions were able to displace intercalatively bound ethidium bromide from ds-DNA. It has been shown that the removal of the N-terminal formamide unit of Dst does not abolish the AT specific mode of binding of the resulting compounds with ds-DNA.[10] We have found that the placement of the positive charge significantly away from the carboxy terminal (as in **11b** in its protonated form, for instance) did not reduce the binding affinity or sequence-specificity.[11] Currently we are examining the DNA binding properties and gene transfer abilities of these systems in detail.

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8. Selected spectroscopic data for compounds **2**–**6**: (**2**) ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm), 0.67 (s, 3H), 0.85–2.24 (complex multiplet, 42H), 3.03 (bs, 2H), 3.12 (s, 6H), 3.36–3.49 (m, 6H), 3.80 (s, 3H), 3.89 (s, 3H), 3.94 (s, 3H), 4.22 (s, 2H), 4.38 (bs, 1H), 5.3 (bs, 1H), 5.54 (bs, 1H), 6.06 (bs, 1H), 6.71 (s, 1H), 6.82 (s, 1H), 7.06 (s, 1H), 7.11 (bs, 1H), 7.37 (s, 1H), 7.43 (s, 1H), 7.54 (bs, 1H), 8.44 (bs, 1H), 8.97 (s, 1H), 9.04 (s, 1H). ESI-MS (+ve ion mode, m/z, %) calcd for $C_{55}H_{84}N_9O_6$: 966.7 (M–Br), obtd. 966.7 (100%). (**3**) ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm), 0.67 (s, 3H), 0.85–2.27 (complex multiplet, 46H), 3.01–3.65 (complex multiplet, 26H), 3.92 (s, 3H), 3.95 (s, 3H), 3.97 (s, 3H), 4.13 (s, 2H), 4.41 (bs, 1H), 5.35 (bs, 2H), 6.09 (bs, 1H), 6.72 (bs, 1H), 6.97 (bs, 1H), 7.08 (bs, 1H), 7.27 (bs, 1H, merging with the $CHCl_3$ peak), 7.46 (bs, 2H), 8.77 (bs, 1H), 8.89 (bs, 1H), 9.39 (bs, 1H).[12] MALDI-TOF (+ve ion mode, m/z, %) calcd for $C_{63}H_{100}N_9O_9$: 1126.9

(M−Br), obtd. 1126. 6 (100%). (**4**) 0.67 (s, 3H), 0.85–2.42 (complex multiplet, 46H), 2.97–3.55 (complex multiplet, 26H), 3.89 (s, 3H), 3.92 (s, 6H), 3.95 (s, 3H), 4.03 (s, 2H), 4.39 (bs, 1H), 5.31 (s, 1H), 5.49 (bs, 1H), 6.04 (bs, 1H), 6.71 (s, 1H), 6.9 (bs, 1H), 6.98 (bs, 1H), 7.09 (s, 1H), 7.24 (s, 1H), 7.33 (s, 1H), 7.44 (s, 1H), 7.48 (s, 1H), 7.54 (s, 1H), 8.64 (bs, 1H), 8.87 (bs, 1H), 9.36 (s, 1H), 9.46 (s, 1H). MALDI-TOF (+ve ion mode, m/z, %) calcd for $C_{69}H_{106}N_{11}O_{10}$: 1249.6 (M−Br), obtd. 1249.9 (100%). (**5**) ^{1}H NMR (300 MHz, $CDCI_3$), δ (ppm), 0.66 (s, 3H), 0.85–2.04 (complex multiplet, 60H), 2.23 (bs, 2H), 2.94–3.40 (m, 14H), 3.88 (s, 3H), 3.91 (s, 3H), 3.96 (s, 3H), 4.21 (s, 2H), 4.43 (bs, 1H), 5.36 (bs, 1H), 5.40 (bs, 1H), 6.08 (bs, 1H), 6.56 (bs, 1H), 6.73 (s, 1H), 6.81 (s, 1H), 7.01 (s, 1H), 7.04 (bs, 1H), 7.29 (s, 1H), 7.35 (s, 1H), 8.56 (bs, 1H), 8.74 (s, 1H), 8.79 (s, 1H). ESI-MS (+ve ion mode, m/z, %) calcd for $C_{65}H_{104}N_9O_6$: 1106.9 (M−Br), obtd. 1106.8 (100%). (**6**) ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm), 0.66 (s, 3H), 0.85–2.04 (complex multiplet, 60H), 2.23 (bs, 2H), 2.94–3.40 (m, 14H), 3.84 (s, 3H), 3.87 (s, 3H), 3.89 (s, 3H), 3.93 (s, 3H), 4.05 (s, 2H), 4.38 (bs, 1H), 5.03 (bs, 1H), 5.38 (bs, 1H), 6.03 (bs, 1H), 6.59 (bs, 1H), 6.69 (s, 1H), 6.82 (s, 1H), 7.08 (bs, 1H), 7.10 (s, 1H), 7.18 (s, 1H), 7.34 (s, 1H), 7.37 (s, 1H), 7.42 (s, 1H), 8.4 (bs, 1H), 8.96 (bs, 2H), 9.19 (bs, 1H). MALDI-TOF (+ve ion mode, m/z, %) calcd for $C_{71}H_{110}N_{11}O_7$: 1229.7 (M−Br), obtd. 1229.7 (100%).

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