Tetrahedron 66 (2010) 1420–1423

Contents lists available at ScienceDirect

Tetrahedron

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

Synthesis of new di- and triamine diosgenin dimers

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ARTICLE INFO

Article history:
Received 30 June 2009
Received in revised form 20 October 2009
Accepted 5 November 2009
Available online 10 November 2009

Keywords:
Dimer
Aldehyde
Diamine
Reductive
Amination
Spacer

ABSTRACT

In this study, a high yield synthesis of symmetrical steroidal polyamine dimers was achieved by the dimerization of (25*R*)-3β-acetoxyfurost-5-en-26-al via several di- and triamine linkers under mild conditions. To ensure the dimerization via E-E ring, the hydroxyl group in diosgenin was protected by an acetyl group. The important step is opening the spiroketal moiety using $NaCNBH_3$/AcOH to furnish the primary alcohol at C-26, followed by oxidation using PCC/CH_2Cl_2 to synthesize the desired aldehyde. Finally, reductive amination with diaminopropane, diaminobutane, diaminohexane, and spermidine using $Na(OAc)_3BH$ as reducing agent, afforded the required four dimers.

1. Introduction

Most DNA-major and minor groove binding molecules possess cationic functional groups, size and shape complementarities to one of the grooves, an aromatic ring system, or combination of these. Steroidal diamines are cationic molecules that have a typical small size aliphatic moiety.[1] The cation type, regiochemical distribution of the ammonium groups, and steroid hydrophobicity may also contribute to the strength and type of steroid–DNA interactions.[2] The binding of spermine and spermidine to DNA is preferentially to the GC-rich major groove and to the AT-rich minor groove regions,[3,4] while hydrophobicity of the steroid surface is important for minor groove recognition and binding. DNA condensation is dependent upon hydrophobicity and distance between positive charges, as well as the total number of charges.[5] The interaction between DNA and steroidal di- or polyamine leads to increase in DNA stability, DNA/RNA discrimination and sequence-selectivity.[6] One of the major sources of steroidal intermediates for the synthesis of steroid hormones and pharmaceutical analogues is Diosgenin that has been reported to have tremendous medical applications. Recently, it has been used to minimize post-menopausal symptoms and has been processed and given to patients to relieve arthritis, asthma, eczema, regulate metabolism, and control fertility. It also provides the steroid building blocks for developing human sex hormones and can be used for developing muscle mass and strength.[7]

In searching for dimeric steroids using diosgenin, few examples were found. The first one is the dimer that was synthesized as a component of artificial lipid bilayer membranes to control their fluidity, and to increase the membranes stability and life time leading to improve their quality by working as a bilayer binder.[8] The dimer was synthesized from diosgenin in 4 steps and 16% overall yield.[9] Moreover, we successfully achieved synthesis of bis-diosgenin pyrazine dimers that represent examples of new cephalostatin analogs.[10] This inspired us to synthesize new dimeric steroids from diosgenin with diamine and triamine as linkers to improve the biological action of these dimers compared with others, which have carbon chains as linkers. These dimers were expected to mimic nature or form a bimolecular sheet for application in the fields of biomimetic and molecular recognition chemistry. In addition, it is expected that their interaction ability with DNA will lead to increase in DNA stability, sequence-selectivity, DNA/RNA discrimination, and hypochromicity of oligonucleotide duplex.

2. Results and discussions

In the present research, we present the synthesis of four dimeric steroids as di- or tri-amino diosgenin dimers. The desired dimers consist of di- or triamine binding two steroid nucleuses via E-E ring linkage type. The dimerization step was achieved through reductive amination reaction between di- or triamine and aldehyde functionality at the steroid E-ring. This functionality obtained from

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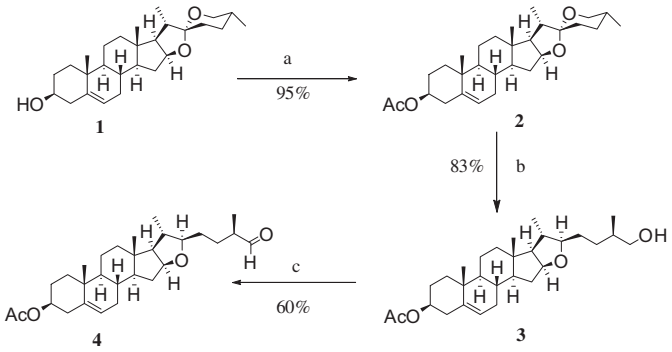
0040-4020/$ – see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.tet.2009.11.037

the oxidation of the hydroxyl group on C-26, which was the product of an F-ring opening reaction of diosgenin acetate **2**. The diosgenin acetate **2** was prepared via esterification reaction of the hydroxyl group at C-3 of diosgenin **1** (Scheme 1). The hydroxyl group in diosgenin **1** was reacted with Ac_2O to furnish in high yield diosgenin acetate **2**.[11] The choice for this group depends on the fact that this group is easy to be removed, and it would not be affected by the chemical transformation in the following steps. The ^1H NMR spectrum confirmed the presence of the acetoxy group at C-3.



Reagents and Conditions: a: Ac_2O, E_3N, DMAP, CH_2Cl_2, 24 h. b: $NaBH_3CN$, AcOH, 48 h. c: PCC, $CaCO_3$, Silica, CH_2Cl_2, 12h.

Scheme 1. Synthesis of (25*R*)-3β-acetoxyfurost-5-en-26-al (**4**).

Next, F-ring opening in diosgenin acetate **2** produced the required hyroxylated steroid. Several reagents have been reported to open the F-ring where Ac_2O/BF_3[12] and $ZnCl_2/Ac_2O$[13] favored E-ring cleavage and Zn/HCl, which gave a triol product.[14] Moreover, $LiAlH_4/AlCl_3$ reductively opened the F-ring with loss of the protecting group at the A-ring to furnish a triol derivative.[9] Our objective was to have only one hydroxyl group at the alkyl chain without opening the E-ring, and without losing the acetate protecting group. This objective was achieved by implementing the methodology reported by Winterfeldt.[11] Therefore, selective F-ring opening was conducted using $NaBH_3CN$ in acetic acid to furnish **3** in good yield after chromatographic separation.

The FTIR spectrum of compound **3** showed a broad peak at 3487 cm^{-1} characteristic of an alcohol group. The ^1H NMR spectra showed a peak at 3.3 ppm (m) for the C-22 proton, which confirmed F-ring opening. In the ^{13}C NMR spectrum, the disappearance of the peak at 109.3 ppm and the appearance of a peak at 90.3 ppm for C-22, further confirmed the fission of the spiroketal moiety. These data were in excellent agreement with the literature values.[11]

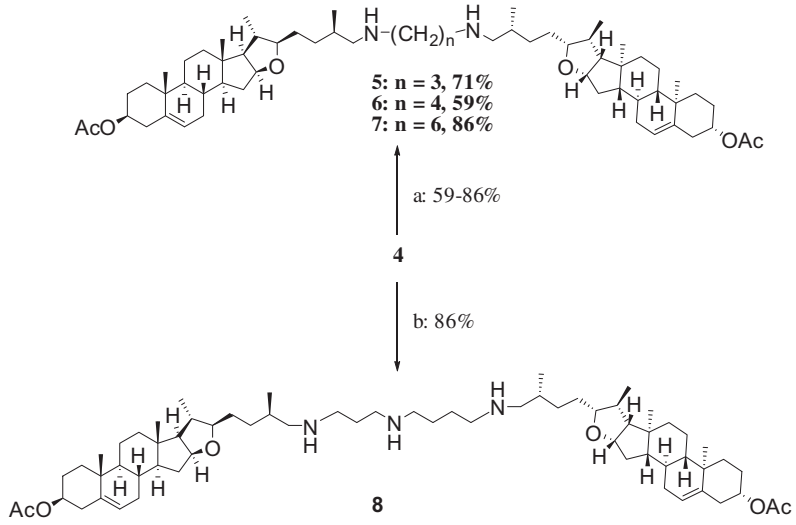
The next step was to produce the aldehyde functionality from the alcohol **3**. Several oxidizing agents could accomplish this goal. However, the most common one is PCC/CH_2Cl_2 in the presence of $CaCO_3$, where $CaCO_3$ was added to moderate the acidic character of PCC. This reagent oxidized the primary alcohol without affecting the acetate group, and it gave compound **4** with an excellent yield. The disappearance of the alcohol peak at 3487 cm^{-1} and the appearance of two peaks for the C-H of the aldehyde functionality, and a peak at 1760 cm^{-1} for C=O stretching. The elemental analysis and the mass (ESI) data were in excellent agreement with the proposed structure for compound **4**.

The final step is the dimerization of the steroidal aldehyde **4** with di- and triamine. This step was achieved using nucleophilic addition of amine followed by reduction of the imine with $NaBH(OAc)_3$ under N_2 gas in dichloroethane (DCE).[15,16,17]

Having the aldehyde **4** in hand, the first dimer **5** was prepared from reductive amination of aldehyde **4** with 1,3-diaminopropane (Scheme 2). The ^1H NMR spectrum showed the presence of peaks at 2.3–2.6 ppm (m) for the 1,3-diaminopropane methylenes protons that are next to nitrogen and for C-26 protons, in addition, the disappearance of the aldehyde proton at 9.7 ppm confirmed the structure of dimer **5**. In the ^{13}C NMR spectrum, the disappearance of the aldehyde peak at 205.3 ppm, and the appearance of a peak at 50.0 ppm for the C–N carbon, also gives a good indication of the structure. The ESI-MS spectrum of dimer **5** showed a molecular ion peak (M+H) at *m*/*z* 955.9, which also confirmed the structure of dimer **5**. The elemental analysis data were in good agreement with the above results and also proved the proposed structure of **5**.

In order to have several dimers with different length spacers to study the structure–activity relationship, two dimers **6** and **7** were prepared in good yields by reductive amination of aldehyde **4** with 1,4-diaminobutane (putrescine) and 1,6-diaminohexane, respectively (Scheme 2).

The FTIR for dimer **6** showed a peak at 3300 cm^{-1} for the amino groups, while the ^1H NMR spectrum exhibited peaks at 2.3–2.8 ppm for the 1,4-diaminobutane methylenes protons that are



Reagents and Conditions: a: 1) 1 equiv of **4** in DCE, AcOH, 1 equiv. of diamine; 2) AcOH, $NaBH(OAc)_3$, 4 days. b: 1) 1 equiv of **4** in DCE, AcOH, 0.5 equiv. of spermidine; 2) AcOH, $NaBH(OAc)_3$, 4 days.

Scheme 2. Synthesis of dimers **5**, **6**, **7** and **8**.

next to nitrogen, and for the C-26 protons. The ESI-MS spectrum, showed a molecular ion peak (M+H) at *m*/*z* 972.1, which proved the molecular weight for dimer **6**. Dimer **7** showed no differences in its FTIR spectrum from that of dimer **6**, however, the ^{1}H NMR spectrum showed peaks at 2.3–2.7 ppm for the 1,6-diaminohexane methylene protons that are next to nitrogen and for the C-26 protons. In addition, the ESI-MS spectrum, showed a molecular ion peak (M+H) at *m*/*z* 999.3, which confirmed the presence of dimer **7**.

Polyamines such as spermidine is widely distributed in nature and display a variety of important biological activities such as protecting DNA against thermal or X-ray induced denaturation, methylation, enzymatic degradation, breakage, and radiation damage.[18] Therefore, we reductively aminated the aldehyde **4** with spermidine and synthesized dimer **8** in 86% yield. The ^{1}H NMR spectrum of dimer **8** exhibited peaks at 2.3–3.1 ppm for the 1,8-diamino-4-azaoctane methylenes protons that are next to nitrogen and for the C-26 protons. In the ESI-MS spectrum, the molecular ion peak appear at *m*/*z* 1026.7, which confirmed the presence of dimer **8**.

3. Conclusion

In order to mimic a bimolecular sheet for application in the fields of biomimetic and molecular recognition chemistry, four new symmetrical steroidal polyamine dimers were designed and synthesized. These dimers were achieved by the dimerization of (25*R*)-3β-acetoxyfurost-5-en-26-al via four di- and triamine linkers under mild conditions and in relatively high yield.

4. Experimental

4.1. General remarks

Melting points (mp) were determined on electrothermal digital melting point apparatus. Materials and reagents were obtained from commercial sources and were used without further purification. FTIR spectra were recorded on a Nicolet-Impact 410 spectrophotometer. Both ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-400 instruments. The chemical shifts (δ) are reported in parts per million relative to TMS used as an internal standard. Mass spectra (MS) were obtained from a LC-MSD-Trap_00125 spectrometer with ESI ion source type. Reactions were monitored by thin layer chromatography (Silica gel 60 F_{254}). Solvents were purified according to the standard.

*4.1.1. Synthesis of (22β,25R)-3β-acetoxyfurost-5-en-26-al, **4**.* PCC (0.7 g, 3.0 mmol) was added to a mixture of powdered $CaCO_3$ (1.3 g, 3.0 mmol), silica (1.0 g), and **3** (2.5 g, 5.5 mmol) in CH_2Cl_2 (50.0 mL) at room temperature. The reaction mixture was stirred for 12 h at room temperature. Then, the reaction mixture was diluted with diethyl ether (50.0 mL) and poured through a short column of Florisil. The solvent was removed under vacuum. Then the residue was purified by column chromatography (10% ethyl acetate/hexane) to give pure product **4** (white solid, 1.5 g, 60%); mp 122–125 °C. FTIR (KBr), ν_{max} 2947, 2893, 2832, 2717, 1736, 1534, 1463, 1380, 1243, 1035 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) selected signals δ: 2.0 (s, 3H, AcO), 3.3 (m, 1H, C-22), 4.3 (m, 1H, C-16), 4.6 (m, 1H, C-3), 5.3 (d, *J*=5.3 Hz, 1H, C-6), 9.7 (d, *J*=2.0 Hz, 1H, C-26). ^{13}C NMR ($CDCl_3$, 100 MHz) selected signals δ: 21.4 (*C*H_3COO), 73.9 (C-3), 83.3 (C-16), 89.7 (C-22), 122.4 (C-6), 139.7 (C-5), 170.6 (CH$_3$*C*OO), 205.3 (C-26). *Anal*. Calcd for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 75.89; H, 9.53.

4.2. General procedure for the dimerization step

*4.2.1. Synthesis of 1,3-bis((22β,25R)-3β-acetoxyfurost-5-en-26-amino)-propane, **5**.* To a solution of **4** (0.3 g, 0.7 mmol) in DCE (10.0 mL), 2.0 mL of glacial AcOH was added, and the mixture was stirred under nitrogen at room temperature for 1 h. 1,3-Diaminopropane (0.078 g, 1.05 mmol) was added and stirred under nitrogen at room temperature for 48 h. $Na(OAc)_3BH$ (0.28 g, 1.3 mmol), then glacial AcOH (0.5 mL) were added and the reaction mixture was stirred under nitrogen for 48 h. The reaction was neutralized with 1 N NaOH and the product was extracted with $CHCl_3$ (2×20.0 mL). The organic layer was washed twice with brine (20 mL), dried over anhydrous Na_2SO_4, and filtered. The solvent was removed under vacuum to yield a yellow oil, which was washed with ether then evaporating under vacuum, to give yellow solid, the solid was purified by preparative TLC (5% ammonia solution/ethanol) to furnish **5** (pale yellow solid, 0.48 g, 71%); mp 71–74 °C. FTIR (KBr), ν_{max} 3459, 2956, 2334, 1734, 1561, 1450, 1377, 1252, 1040 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) selected signals δ: 2.0 (s, 3H, AcO), 2.3–2.6 (m, 4H, methylenes next to nitrogen), 3.3 (m, 1H, C-22), 4.3 (m, 1H, C-16), 4.6 (m, 1H, C-3), 5.4 (d, *J*=4.5 Hz, 1H, C-6). ^{13}C NMR ($CDCl_3$, 100 MHz) selected signals δ: 21.5 (*C*H_3COO), 50.0 (C–N), 73.9 (C-3), 83.2 (C-16), 90.5 (C-22), 122.4 (C-6), 139.7 (C-5), 170.6 (CH$_3$*C*OO). M.wt calcd for $C_{61}H_{98}N_2O_6$: 955.4 g/mol. Found MS (ESI), *m*/*z* (relative intensity): 955.9 (M^+, 39), 557.5 ($C_{35}O_3N_2H_{59}^+$, 100). *Anal*. Calcd for $C_{61}H_{98}N_2O_6$: C, 76.68; H, 10.34; N, 2.93. Found: C, 75.48; H, 9.71; N, 3.38.

*4.2.2. Synthesis of 1,4-bis((22β,25R)-3β-acetoxyfurost-5-en-26-amino)-butane, **6**.* Pure dimer **6** (pale yellow solid, 0.40 g, 59%) was prepared utilizing the general procedure, through the reaction of **4** (0.3 g, 0.7 mmol), 1,4-diaminobutane (0.09 g, 1.05 mmol) and $Na(OAc)_3BH$ (0.28 g, 1.3 mmol); mp 95–98 °C (Decomposition). FTIR (KBr) ν_{max}: 3377, 2943, 2358, 1662, 1598, 1449, 1235, 1053 cm^{-1}. ^{1}H NMR (ppm) selected signals δ: 2.0 (s, 3H, AcO), 2.3–2.9 (m, 4H, methylenes next to nitrogen), 3.3 (m, 1H, C-22), 4.3 (m, 1H, C-16), 4.6 (m, 1H, C-3), 5.4 (br s, 1H, C-6). ^{13}C NMR (ppm) selected signals δ: 21.3 (*C*H_3COO), 50.0 (C–N), 73.8 (C-3), 83.3 (C-16), 90.2 (C-22), 122.1 (C-6), 139.7 (C-5), 170.5 (CH$_3$*C*OO). M.wt calcd for $C_{62}H_{100}N_2O_6$: 968.8 g/mol. Found MS (ESI), *m*/*z* (relative intensity): 972.1 (M^++2, 100), 660.9 (81), 611.9 ($C_{39}H_{68}N_2O_3^+$, 33).

*4.2.3. Synthesis of 1,6-bis((22β,25R)-3β-acetoxyfurost-5-en-26-amino)-hexane, **7**.* Pure dimer **7** (pale yellow solid, 0.60 g, 86%) was prepared utilizing the general procedure, through the reaction of **4** (0.3 g, 0.7 mmol), 1,6-diaminohexane (0.12 g, 1.05 mmol) and $Na(OAc)_3BH$ (0.28 g, 1.3 mmol); mp 67 °C (Decomposition). FTIR (KBr), ν_{max} 3467, 2942, 2367, 1747, 1572, 1424, 1243, 1024 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) selected signals δ: 2.0(s, 3H, AcO), 2.2–2.7 (m, 4H, methylenes next to nitrogen), 3.3 (m, 1H, C-22), 4.3 (dt, *J*=13.0, 7.5 Hz, 1H, C-16), 4.6 (m, 1H, C-3), 5.3 (d, *J*=4.3 Hz, 1H, C-6). ^{13}C NMR ($CDCl_3$, 100 MHz) selected signals δ: 21.4(*C*H_3COO), 50.0 (C–N), 73.8 (C-3), 83.1 (C-16), 90.4 (C-22), 122.3 (C-6), 139.6 (C-5), 170.5 (CH$_3$*C*OO). M.wt calcd for $C_{64}H_{104}N_2O_6$: 997.5 g/mol. Found MS, *m*/*z* (relative intensity): 999.3 (M^++1, 32). *Anal*. Calcd for $C_{64}H_{104}N_2O_6$: C, 77.06; H, 10.51; N, 2.81. Found: C, 76.70; H, 10.09; N, 2.49.

*4.2.4. Synthesis of 1,8-bis((22β,25R)-3β-acetoxyfurost-5-en-26-amino)-4-azaoctane, **8**.* Pure dimer **8** (pale yellow solid, 0.62 g, 86%) was prepared utilizing the general procedure, through the reaction of **4** (0.3 g, 0.7 mmol), 1,8-diamino-4-azaoctane (0.15 g, 1.05 mmol) and $Na(OAc)_3BH$ (0.28 g, 1.3 mmol); mp 227–230 °C (Decomposition). FTIR (KBr), ν_{max} 3480, 2954, 2356, 1737, 1574, 1444, 1248, 1139 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) selected signals δ: 2.0 (s, 3H, AcO), 2.3–3.0 (m, methylenes next to nitrogen), 3.3 (m, 1H, C-22), 4.3 (m, 1H, C-16), 4.6 (m, 1H, C-3), 5.4 (d, *J*=4.5 Hz, 1H, C-6). ^{13}C NMR ($CDCl_3$, 100 MHz) selected signals δ: 21.6 (*C*H_3COO), 50.3 (C–N), 74.4 (C-3), 83.5 (C-16), 90.2 (C-22), 123.4 (C-6), 139.9 (C-5), 171.3 (CH$_3$*C*OO). M.wt calcd for $C_{65}H_{107}N_3O_6$: 1026.6 g/mol. Found MS, *m*/*z* (relative

intensity): 1026.7 (M^+, 19), 630.0 ($C_{39}H_{68}N_3O_3^+$, 64), 689.5 ($C_{36}H_{63}N_3O_3^+$, 100). *Anal.* Calcd for $C_{65}H_{107}N_3O_6$: C, 76.05; H, 10.51; N, 4.09. Found: C, 76.37; H, 9.98; N, 3.91.

Acknowledgements

This research is supported by the Deanship of Research, Jordan University of Science and Technology.

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