A-ring functionalization of cholestane with highly substituted pyrans and 2-aminoisophthalonitriles

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Dedicated to Prof. Jan Bergman on the occasion of his 80th anniversary

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Abstract

Following our ongoing interest on modified steroidal compounds for biological purposes, herein we endeavor the two-step regioselective synthesis of three-substituted pyran-cholestane fused compounds (6 examples, yields up to 83%) at C-2:C-3 A-ring side of the steroid scaffold. In the presence of higher amounts of malononitrile, the intermediate α,β-unsaturated carbonyl substrates give rise to competing reactions leading to the formation of the corresponding 2-aminoisophthalonitrile fused compounds (2 examples, up to 37% yield).

Keywords: Steroids, cholestane, substituted pyrans, 2-aminoisophthalonitriles, hybridization

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Introduction

Steroid compounds are widely present in living organisms playing important roles in their vital activities. The most recognizable steroid is cholesterol (cholest-5-en-3β-ol), which is considered a lipid-type molecule, being one of the most important structural components of cell membranes. Cholesterol is a rigid and almost planar molecule with a steroidal skeleton of four fused rings, three six-membered and one five-membered, conventionally lettered from A to D (1,2-cyclopentanoperhydrophenanthrene ring system) (Figure 1).

![Figure 1](image1.png)

Figure 1. (A) Numbering and ring-labeling convention in cholesterol; (B) four domains of cholesterol.

The cholesterol molecule contains four essential domains (Figure 1B). In domain I, the polarity of the 3-hydroxy group constitutes an active site for hydrogen bond interactions with a variety of biological molecules. In domain II, the absence of methyl groups at C-4 and C-14 influences directly the planarity of the molecule, while in domain III, the natural (R)-configuration at C-20 determines the “right-handed” conformation of the side chain. Finally, in domain IV, the conformation and length of the side chain is of prime relevance to intermolecular contacts. The unique properties of cholesterol make it a very attractive molecule for synthetic organic studies, as well as to other interesting applications such as drug delivery and bioimaging applications, and even liquid crystals and gelators. A key structural feature is the presence of the hydrophilic 3-hydroxy headgroup on the A-ring, together with a hydrophobic hydrocarbon body, which offer the molecule an amphiphilic nature, and revealed itself as being an important property in the interaction with protein aggregates. In fact, lanosterol and 25-hydroxycholesterol (Figure 2) were reported to reverse crystallin-induced protein aggregation, by dissolving aggregated proteins from amyloid-like fibrils. Due to their amphiphilic nature, they were able to intercalate and coat hydrophobic core areas of large protein aggregates, allowing these aggregates to become water-soluble again. The efficacy of lanosterol to reverse multiple types of mutant-crystallin aggregates was further improved trough 2-fluorination and 25-hydroxylation, enhancing the anti-aggregating activity (Figure 2). Remarkably, lanosterol is also capable to inhibit the self-assembly of Aβ peptides, entangling with peptides and forming a hydrophobic core with residues Phe-19 and Phe-20.

![Figure 2](image2.png)

Figure 2. Chemical structures of lanosterol, 25-hydroxycholesterol, and 2-fluoro-25-hydroxylanosterol.
The improvement of biological properties of natural occurring lanosterol by chemical modification is a critical example of how the right chemical modification in a natural scaffold could lead to highly efficient molecules for therapeutic purposes. Over the years, steroid group compounds, particularly cholesterol, have played a significant interest for organic synthesis, due to good availability, low cost and easily derivatization of the functional groups. Many useful chemical and enzymatic reactions are now widely used for multi-step steroid transformations, leading to important products.\(^3\)\(^8\) The chemical transformations range from simple ones, like manipulations of functional groups, to more complex ones such C-H activation or C–C bonds formation with organometallic reagents.\(^8\) From the literature analysis one can realize that highly substituted pyrans tethered with \(-\text{NH}_2\) and \(-\text{CN}\) functionalities at C-2 and C-3 positions (Figure 3), have important pharmacological properties.\(^9\)\(^-\)\(^11\) Furthermore, it should be highlighted the significance of the 2-amino-3-cyano-4-phenylpyran pharmacophoric fragment (highlighted in red) in AChE inhibitors\(^12\) or even as anticancer\(^13\) and antibacterial\(^14\) molecules (Figure 3).

![Figure 3. A-ring fused pyran derivatives.](image)

Following our interest in modified steroidal compounds with biological applications, we have looked at the chemical modification of cholestan A-ring, by following a two-step synthetic procedure in order to reach a three-substituted fused pyran heterocyclic ring as depicted in Figure 3. From the retrosynthetic analysis, one can expect that the initial aldol reaction can occur at C-2 or C-4 positions of the steroid scaffold (Figure 4). The selectivity towards one particular position will allow the selective fusion of pyran ring at one specific A-ring side (Figure 4). To the best of our knowledge, herein we present for the first time the A-ring functionalization of cholestan scaffold with highly substituted pyrans tethered with \(-\text{NH}_2\) and \(-\text{CN}\) functionalities (Figure 4).

![Figure 4. Retrosynthetic analysis of the two-step synthetic methodology. Regioselective functionalization of C-2:C-3 A-ring side of cholestan.](image)
Results and Discussion

The planned synthetic strategy for the preparation of pyran-fused cholesterol derivatives 4a-f encompasses two reaction steps: 1) the aldol reaction of cholesstan-3-one 1 and a series of benzaldehydes 2a-f to give the intermediate α,β-unsaturated carbonyl derivatives 3a-f; and 2) the microwave (MW)-assisted cyclization reaction of α,β-unsaturated carbonyl derivatives 3a-f and malononitrile (Scheme 1). The first reaction step afforded the α,β-unsaturated carbonyl 3a-f (arbitrary numbering for NMR analysis, Figure 5) in good yields (70-80%), in the presence of t-BuOK (5 equiv) as base. The reaction was quite selective towards the C-2 position of the steroid scaffold (see 1H and 13C NMR spectra in SI) (Scheme 1). As we used an excess of benzaldehydes 2a-f (3 equiv), it was expected to have competition between C-2 and C-4 positions, however, no C-4 reaction products were detected in the reaction media. Possibly this might be due to the more steric hindrance of carbon at position 4 of the steroid scaffold.

![Scheme 1. Synthetic strategy for the two-step preparation of pyran-fused cholesterol derivatives 4a-f.](image)

The second step of the synthetic strategy was not straightforward, and optimization of the reaction conditions was required. To do so, we used the 4-CH3 α,β-unsaturated carbonyl derivative 3b as model substrate and the outcome of the optimization study is summarized in Table 1. In the first attempts, we evaluated the effect of increasing amounts of malononitrile (1 to 5 equiv), ending up with increasing yields (up to 75%) of the desired pyran derivative 4b (mixture of two diastereomers, Figure 5) (Table 1, entries 1-3). When the amount of malononitrile was further increased to 10 and 20 equiv, the formation of 2-aminoisophthalonitrile derivative 5b (blue inset) was observed in 30 and 33% yields, respectively, with a consequent decrease in the obtained yield of the respective pyran derivative 4b (Table 1, entries 4 and 5). Changing the substituent from R1 = CH3 to R1 = OCH3 no better improvements were observed and the formation of 2-aminoisophthalonitrile 5c took place in 30-37% yields (Table 1, entries 4-7). Then it can be concluded that the formation of 2-aminoisophthalonitriles 5 depends on the amount of malononitrile used in the reaction. The synthesis of 2-aminoisophthalonitriles 5, could be explained on the basis of the following mechanism: i) Michael-addition of malononitrile to the β-position of α,β-unsaturated carbonyl derivative 3, followed by 1,2-addition of a second molecule of malononitrile and dehydration to give intermediate III (Scheme 2); ii) base-catalyzed intramolecular attack to cyanide group to give intermediate IV, followed by a [1,5] proton shift giving V (Scheme 2); iii) HCN elimination ending up with the formation of 2-aminoisophthalonitriles 5 (Scheme 2).

With the adequate amount of malononitrile well established in order to drive the reaction towards the desired pyran derivatives 4, we also struggled to further improve the yield of pyran derivative 4b. Increasing
amounts of DBU (10 to 30 mol%) were attempted, as well other base-catalysts such as Et$_3$N and K$_2$CO$_3$ (Table 1, entries 8-11). The isolated yield of pyran 4b remained unaffected with the increasing amounts of DBU (Table 1, entries 3, 8 and 9), and the switch from DBU to Et$_3$N and K$_2$CO$_3$, ended up with a slight decrease in the isolated yield of 4b (Table 1, entries 10 and 11). The screening of the reaction conditions indicated that the higher possible yield for derivative 4b (75%) is obtained using 5 equiv of malononitrile, in the presence of 10 mol% of DBU (Table 1, entry 3). Therefore, these reaction conditions were further applied to the remaining α,β-unsaturated carbonyl derivatives 3a-f, giving the corresponding pyran derivatives 4a-f in 65-83% yield (Table 1, entries 3, 12-16).

Figure 5. Arbitrary numbering of compounds 3a-f, 4a-f and 5a,b for NMR analysis and characterization.

Scheme 2. Plausible mechanism for the formation of 2-aminoisophthalonitrile derivatives 5b,c.
Table 1. Reaction conditions screening and reaction scope for the synthesis of derivatives 4a-f

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<tr>
<th>Entry</th>
<th>Derivative (R)</th>
<th>Reaction conditions</th>
<th>4 Yield (%)[b]</th>
<th>5 Yield (%)[b]</th>
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<table>
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<tr>
<th>Entry</th>
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<th>Reaction conditions</th>
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<th>5 Yield (%)[b]</th>
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<td>16</td>
<td>3f (3,5-OCH₃)</td>
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[a] Reactions carried out using 0.1 mmol of 3, in EtOH (2 mL) as solvent, under MW-irradiation at 80 °C for 20 min; [b] isolated yield.

Conclusions

A series of six pyran-fused cholestane derivatives 4a-f was synthesized in good yields, exploring the reaction of α,β-unsaturated carbonyl compounds 3a-f with malononitrile, in the presence of DBU. The initial aldol reaction step of cholestan-3-one with benzaldehydes was regioselective towards C-2 position, which is the less hindered position of the steroid scaffold. The two-step synthetic methodology, rather than the one-pot procedure, takes advantage of the aldol reaction regioselectivity, granting the selective fusion of the three-substituted pyran ring at the C2-C3 positions of cholestane A-ring. In the presence of a high excess of malononitrile, there is the formation of 2-aminoisophthalonitrile fused to C2-C3 of the cholestane A-ring. This is a competing side reaction when higher amounts of malononitrile are input into the reaction.
Experimental Section

General. All chemicals were commercially available except those having the synthesis described. All reaction mixtures were monitored by thin layer chromatography (TLC) using commercial TLC plates (Merck Kieselgel 60 F254). The plates were observed under UV light at 254 and 365 nm. Melting points were determined using a Reichert Thermovar apparatus fitted with a microscope and are uncorrected. NMR spectra were recorded in Bruker Avance 300 spectrometer (300 MHz for 1H and 75 MHz for 13C), in CDCl₃ as solvent, if not stated otherwise. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz; internal standard was residual peak of the solvent. Unequivocal 13C assignments were made with the aid of 2D gHSQC and gHMBC (delays for one- bond and long-range J C/H couplings were optimised for 145 and 7 Hz, respectively) experiments. High-resolution mass spectra analysis (HRMS-ESI) were performed on a microTOF (focus) mass spectrometer. Ions were generated using an Apollol (ESI) source. Ionization was achieved by electrospray, using a voltage of 4500 V applied to the needle, and a counter voltage between 100 and 150 V applied to the capillary.

General procedure for the synthesis of α,β-unsaturated carbonyl compounds (3a-f). To a stirring solution of cholestan-3-one 1 (200 mg, 0.5 mmol) and t-BuOK (290 mg, 2.5 mmol) in refluxing EtOH (5 mL), was added the appropriate benzaldehyde 2a-f (1.5 mmol). The resulting reaction mixture was kept under reflux for 2-3 h. After completion of the reaction (TLC) the mixture was poured into cold water, the pH adjusted to 4 with 10% aqueous HCl and the resulting precipitate recovered by filtration. The crude precipitate was dissolved in CH₂Cl₂ (10 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was then purified by preparative TLC using hexane:ethyl acetate (10:1) as eluent.

**55,8R,9S,10S,13R,14S,17R**-2-[[(E)-Benzyldene]-10,13-dimethyl-17-[(R)-6-methylheptan-2-yl]hexadecahydro-3H-cyclopenta[a]phenanthren-3-one (3a). (172 mg, 70%), mp 97-99 °C. 1H-NMR (300 MHz, CDCl₃): δ = 0.65 (s, 3H, 18- or 19-CH₃), 0.79 (s, 3H, 18- or 19-CH₃), 0.85-2.29 (m, 36H), 2.45 (dd, 1H, H-4, J 18.6, 5.4 Hz), 3.11 (d, 1H, H-1, J 15.8 Hz), 7.31-7.41 (m, 5H, H-2',3',4',5',6'), 7.55-7.56 (m, 1H, H-β) ppm. 13C-NMR (75 MHz, CDCl₃): δ = 11.9, 12.0, 18.7, 21.4, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.7, 31.5, 35.4, 35.9, 36.2, 39.5, 42.0, 42.3, 42.5, 42.8, 53.6, 56.3, 56.4, 128.4 (C-2',6'), 128.5 (C-4'), 130.3 (C-3',5'), 135.4 (C-1'), 135.7 (C-2), 137.1 (C-β), 201.8 (C-3) ppm. HRMS (ESI¹⁺): m/z [M+H]⁺ calcd for C₃₄H₅₅O: 475.3934; found 475.3960.

**55,8R,9S,10S,13R,14S,17R**-10,13-Dimethyl-2-[[(E)-4-methylenebenzylidene]-17-[(R)-6-methylheptan-2-yl]hexadecahydro-3H-cyclopenta[a]phenanthren-3-one (3b). (202 mg, 80%), mp 149-151 °C. 1H-NMR (300 MHz, CDCl₃): δ = 0.65 (s, 3H, 18- or 19-CH₃), 0.78 (s, 3H, 18- or 19-CH₃), 0.85-2.27 (m, 36H), 2.37 (s, 3H, 4'-CH₃), 2.44 (dd, 1H, H-4, J 18.7, 5.4 Hz), 3.10 (d, 1H, H-1, J 15.8 Hz), 7.20 (d, 2H, H-3',5', J 8.1 Hz), 7.30 (d, 2H, H-2',6', J 8.1 Hz), 7.54-7.55 (m, 1H, H-β) ppm. 13C-NMR (75 MHz, CDCl₃): δ = 11.9, 12.0, 18.7, 21.41, 21.44, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.7, 31.5, 35.4, 35.9, 36.3, 39.5, 40.0, 42.0, 42.3, 42.5, 42.8, 53.6, 56.3, 56.4, 129.1 (C-3',5'), 130.5 (C-2',6'), 132.9 (C-1'), 134.6 (C-2), 137.3 (C-β), 138.8 (C-4'), 201.7 (C-3) ppm. HRMS (ESI¹⁺): m/z [M+H⁺]⁺ calcd for C₃₅H₅₃O: 489.4091; found 489.4098.

**55,8R,9S,10S,13R,14S,17R**-2-[[(E)-4-Methoxybenzylidene]-10,13-dimethyl-17-[(R)-6-methylheptan-2-yl]hexadecahydro-3H-cyclopenta[a]phenanthren-3-one (3c). (209 mg, 80%), mp 74-76 °C. 1H-NMR (300 MHz, CDCl₃): δ = 0.65 (s, 3H, 18- or 19-CH₃), 0.79 (s, 3H, 18- or 19-CH₃), 0.85-2.27 (m, 36H), 2.43 (dd, 1H, H-4, J 18.7, 5.4 Hz), 3.08 (d, 1H, H-1, J 16.1 Hz), 3.84 (s, 3H, 4'-OC₃H₃), 6.93 (d, 2H, H-3',5', J 8.8 Hz), 7.38 (d, 2H, H-2',6', J 8.8 Hz), 7.54-7.55 (m, 1H, H-β) ppm. 13C-NMR (75 MHz, CDCl₃): δ = 11.9, 12.0, 18.7, 21.5, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.7, 31.5, 35.4, 35.8, 36.2, 39.5, 40.0, 42.1, 42.5, 42.7, 53.7, 55.3, 56.3, 56.4, 113.9 (C-3',5'), 128.3 (C-1'), 132.3 (C-2',6'), 133.3 (C-2), 137.2 (C-β), 159.9 (C-4'), 201.5 (C-3) ppm. HRMS (ESI¹⁺): m/z [M+H⁺]⁺ calcd for C₃₅H₅₃O₂: 505.4040; found 505.4040.
(55,8R,9S,10S,13R,14S,17R)-2-[(E)-4-(Dimethylamino)benzylidene]-10,13-dimethyl-17-[(R)-6-methylheptan-2-yl]hexadecahydro-3H-cyclopenta[a]phenanthen-3-one (3d).  (187 mg, 70%). mp 167-168 °C.  1H-NMR (300 MHz, CDCl3): δ = 0.66 (s, 3H, 18- or 19-CH3), 0.79 (s, 3H, 18- or 19-CH3), 0.86-2.27 (m, 36H), 2.41 (dd, 1H, H-4, J 18.9, 5.5 Hz), 3.02 [s, 6H, 4’-N(CH3)2], 3.09 (d, 1H, H-1, J 15.7 Hz), 6.71 (d, 2H, H-3’, 5’, J 8.7 Hz), 7.40 (d, 2H, H-2’, 6’, J 8.7 Hz), 7.58-7.59 (m, 1H, H-β) ppm. 13C-NMR (75 MHz, CDCl3): δ = 12.0, 18.7, 21.5, 22.6, 22.8, 23.9, 24.3, 28.0, 28.3, 28.7, 31.6, 35.4, 35.6, 35.8, 36.2, 39.5, 40.0, 40.1, 41.9, 42.5, 53.8, 56.3, 56.4, 111.6 (C-3’,5’), 123.6 (C-1’), 130.5 (C-2’), 132.7 (C-2’, 6’), 138.5 (C-β), 150.5 (C-4’), 201.1 (C-3) ppm. HRMS (ESI+): m/z [M+H]+ calcd for C36H56ON: 518.4356; found 518.4357.

(55,8R,9S,10S,13R,14S,17R)-2-[(E)-3,4-Dimethoxybenzylidene]-10,13-dimethyl-17-[(R)-6-methylheptan-2-yl]hexadecahydro-3H-cyclopenta[a]phenanthen-3-one (3e).  (207 mg, 75%). mp 58-61 °C.  1H-NMR (300 MHz, CDCl3): δ = 0.66 (s, 3H, 18- or 19-CH3), 0.80 (s, 3H, 18- or 19-CH3), 0.85-2.28 (m, 36H), 2.44 (dd, 1H, H-4, J 18.8, 5.4 Hz), 3.13 (d, 1H, H-1, J 15.8 Hz), 3.89 (s, 3H, 3’- or 4’-OCH3), 3.92 (s, 3H, 3’- or 4’-OCH3), 6.90 (d, 1H, H-5’, J 8.4 Hz), 6.96 (d, 1H, H-2’, J 2.0 Hz), 7.05 (dd, 1H, H-6’, J 8.4, 2.0 Hz), 7.53-7.54 (m, 1H, H-β) ppm. 13C-NMR (75 MHz, CDCl3): δ = 12.0, 18.7, 21.5, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.7, 31.5, 35.4, 35.8, 36.1, 39.5, 39.9, 42.1, 42.2, 42.5, 42.7, 53.7, 55.85, 55.90, 56.29, 56.35, 110.8 (C-5’), 113.7 (C-2’), 123.9 (C-6’), 128.6 (C-1’), 133.5 (C-2), 137.4 (C-β), 148.5 (C-3’), 149.5 (C-4’), 201.4 (C-3) ppm. HRMS (ESI+): m/z [M+H]+ calcd for C36H55O3: 535.4146; found 535.4160.

General procedure for the pyran-fused compounds (4a-f). The appropriate α,β-unsaturated carbonyl compound 3a-f (0.1 mmol), malononitrile (33mg, 0.5 mmol), DBU (10 mol%) and EtOH (2 mL) were mixed in a CEM Microwave tube. The resulting mixture was heated at 80 °C for 20 min under MW-irradiation. After that period, the solvent was removed under reduced pressure, the crude product dissolved in CH2Cl2 (5 mL) and purified by preparative TLC, using a 2:1 mixture of hexane:ethyl acetate as eluent. The compounds were obtained as a mixture of two diastereomers, arbitrary assigned as d1 and d2, from the analysis of 1H, 13C NMR, HSQC and HMBC spectra.

Pyran compound (4a) (mixture of d1 and d2 diastereomers) = (1R,3aS,3bR,5aS,10R/S,11aS,11bS,13aR)-8-amino-11a,13a-dimethyl-1-[(R)-6-methylheptan-2-yl]-10-phenyl-1,2,3,3a,3b,4,5,5a,6,10,11,11a,11b,12,13,13a-hexadecahydrocyclopenta[5,6]naphtho[1,2-g]chromene-9-carbonitrile.  (42 mg, 78%).  1H-NMR (300 MHz, CDCl3): δ = 0.55-2.08 (m, 88H, d1+d2), 3.75 (s, 2H, H-1’, d1+d2), 4.33 (s, 2H, 3’-NH2, d2), 3.74-7.35 (m, 10H, H-2’,, 3’, 4’, 5’, 6’, d1+d2) ppm. 13C-NMR (75 MHz, CDCl3): δ = 11.5, 11.9, 11.96, 11.98, 18.6, 21.1, 21.6, 22.7, 22.8, 23.8, 23.9, 24.2, 28.0, 28.2, 30.4, 30.5, 31.5, 35.0, 35.3, 35.36, 35.40, 35.7, 35.8, 36.1, 39.5, 39.8, 39.9, 41.5, 41.6, 42.0, 42.1, 42.4, 43.5, 53.2, 53.5, 56.2, 60.9, 108.1 (C-2, d1), 108.5 (C-2, d2), 120.2 (CN, d1), 120.3 (CN, d2), 127.1 (C-4’), 128.7 (C-2’, 6’, d1), 127.9 (C-2’, 6’, d2), 128.5 (C-3’, 5’, d2), 128.6 (C-3’, 5’, d2), 141.5 (C-1’), 141.6 (C-1’), d1), 141.6 (C-1’, d2), 143.1 (C-3, d1), 143.3 (C-3, d2), 159.0 (C-3’, d1+d2) ppm. HRMS (ESI+): m/z [M+H]+ calcd for C37H53N2O: 541.4152; found 541.4153.
Pyran compound (4b) (mixture of d1 and d2 diastereomers) = (1R,3aS,3bR,5aS,10R/S,11aS,11bS,13aR)-8-amino-11a,13a-dimethyl-1-[(R)-6-methylheptan-2-yl]-10-(4-methylphenyl)-1,2,3,3a,3b,4,5,5a,6,10,11,11a,11b,12,13,13a-hexadecahydrocyclopenta[5,6]naphtho[1,2-g]chromene-9-carbonitrile.

Pyran compound (4c) (mixture of d1 and d2 diastereomers) = (1R,3aS,3bR,5aS,10R/S,11aS,11bS,13aR)-8-amino-10-(4-methoxyphenyl)phenyl-11a,13a-dimethyl-1-[(R)-6-methylheptan-2-yl]-1,2,3,3a,3b,4,5,5a,6,10,11,11a,11b,12,13,13a-hexadecahydrocyclopenta[5,6]naphtho[1,2-g]chromene-9-carbonitrile.

Pyran compound (4d) (mixture of d1 and d2 diastereomers) = (1R,3aS,3bR,5aS,10R/S,11aS,11bS,13aR)-8-amino-10-[(4-(dimethylamino)phenyl)phenyl-11a,13a-dimethyl-1-[(R)-6-methylheptan-2-yl]-1,2,3,3a,3b,4,5,5a,6,10,11,11a,11b,12,13,13a-hexadecahydrocyclopenta[5,6]naphtho[1,2-g]chromene-9-carbonitrile.
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Supplementary Material

$^1$H-, $^{13}$C-NMR and HRMS spectra for all new compounds are available in the supplementary file accompanying this paper.

References


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