Tetrahedron 64 (2008) 5298–5305

Contents lists available at ScienceDirect

Tetrahedron

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

Synthesis of diamino-furostan sapogenins and their use as scaffolds for positioning peptides in a preorganized form

Daniel G. Rivera [a,*], Odette Concepción [a], Karell Pérez-Labrada [b], Francisco Coll [a]

[a] Center for Natural Products Study, Faculty of Chemistry, University of Havana, 10400 Havana, Cuba
[b] Department of Chemistry, Institute of Pharmacy and Food, University of Havana, 10400 Havana, Cuba

ARTICLE INFO

Article history:
Received 20 February 2008
Accepted 7 March 2008
Available online 12 March 2008

Dedicated to Professor José Luis Mola Garate on the occasion of his 75th birthday

Keywords:
Steroids
Steroidal sapogenins
Furostanes
Peptides
Cyclopeptides

ABSTRACT

The synthesis of peptide–furostane conjugates from natural steroidal sapogenins is reported. The approach comprises the introduction of α-oriented amino groups into spirostanic sapogenins followed by reductive opening of the spiroketal chain, thus producing diamino-furostanic scaffolds suitable for further functionalization. Solid and solution-phase coupling processes were utilized for the incorporation of various α-amino acids and peptides into the furostanic skeletons. The attachment position depends on the steroidal sapogenin originally used, i.e., diosgenin or hecogenin. The resulting furostanic skeletons feature a trans A/B-ring fusion and hold the peptides in axial disposition. This characteristic ensures a preorganized alignment of the peptidic motifs, an important structural feature for future applications in molecular recognition and catalysis. A macrocyclic tripeptide–furostane conjugate was also produced by a combination of peptide coupling, Staudinger ligation, and a cyclization protocol. This work constitutes the first report on the use of furostanic sapogenins as scaffolds for positioning natural amino acids and (cyclo)peptides.

1. Introduction

The use of preorganized scaffolds to achieve a specific alignment of peptide chains has proven to be a success over the years. Such organizing frameworks have been of great importance either for studying various types of peptide folding or for recognition studies of amino acids, peptides, and proteins.[1] Among the architectures used, cyclotriveratrylenes,[2] calix[4]arenes,[3] trialkylbenzenes,[4] Kemp's triacid,[5] and steroidal skeletons[6] have found the greatest applicability due to their ability to align the pedant peptide chains in a preorganized form. The steroidal nucleus is considered as a privileged scaffold for this approach, as it combines chirality, high availability, and various fuctionalization patterns that can be modified in a tunable manner.

The steroidal nucleus of greater relevance on this field is the cholanic skeleton of bile acids.[6,7] The value of this scaffold arises mainly from its curved, concave-shaped structure that can be functionalized to achieve an appropriate array of binding elements. For example, libraries of peptide–steroid conjugates have been prepared from suitable amino-cholanes functionalized on the α face.[6a–e] Some of these properly aligned peptide fragments have proved to be useful as artificial enzymes (e.g., serine-like proteases),[6d] for binding studies of specific oligopeptides sequences (e.g., opioid peptides),[6g,h] and for imposing facial amphiphilicity into the steroidal conjugates.[6b] Also, hybrid peptide–cholane macrocycles have been synthesized as a mean to force the peptide chains upon folded structures.[8,6f] This approach has demonstrated that the conjugation of peptides to rigid steroidal scaffolds combined with cyclization is a promising idea toward the production of novel structures and properties.[9]

Whereas the cholanic skeleton of bile acids is naturally endowed with a cis A/B-ring fusion (i.e., 5β-configuration), this feature has been eventually modified to achieve different alignments of the pendant arms.[6g,10] In terms of conformational constrain, the advantage of employing an A/B-trans steroidal nucleus containing all functionalities with axial orientation has been well documented.[10] For this, an alternative to the manipulation of bile acids is the use of steroids featuring a natural trans A/B-ring fusion and being suitably

Abbreviations: Boc, *tert*-butoxycarbonyl; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; DCC, *N,N′*-dicyclohexylcarbodiimide; DIC, *N,N′*-diisopropylcarbodiimide; DIPEA, *N,N*-di-*iso*-propyl-*N*-ethylamine; DMAP, *N,N*-dimethylaminopyridine; DMF, dimethylformamide; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidonone; HATU, *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxy-1*H*-benzotriazole; PCC, pyridinium chlorochromate; TBHP, *tert*-butyl hydroperoxide; TBAB, tetra-*n*-butyl ammonium bromide; TBDPS, *tert*-butyldiphenylsilyl; TFA, trifluoroacetic acid.

* Corresponding author. Tel.: +53 7 8702102; fax: +53 7 8733502.
E-mail address: dgr@fq.uh.cu (D.G. Rivera).

0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.03.023

functionalized at various positions. This is the case of some steroidal sapogenins, which comprise both amenable functionalization patterns and accessible 5α-skeletons.

Herein, we report on the use of furostan sapogenins as scaffolds for positioning peptides in a preorganized form. Steroidal sapogenins are natural steroids traditionally used in the production of steroidal hormones[11] and the synthesis of bioactive natural products (analogs).[12] Several sapogenins are readily available materials with functionalities that can be easily converted into those required for peptide synthesis by a sort of standard procedures. Furostan sapogenins can be obtained straightforwardly from available spirostan sapogenins by reductive opening of the spiroketal moiety,[13] a procedure that renders a hydroxy-functionalized side chain suitable to attach to a solid support. Thus, the furostanic skeleton can be considered as an alternative A/B-trans scaffold for positioning axially-disposed peptide chains. The approach includes the preparation of diamino-furostanes and their subsequent use on the solid and solution-phase syntheses of peptide–furostane conjugates.

2. Results and discussion

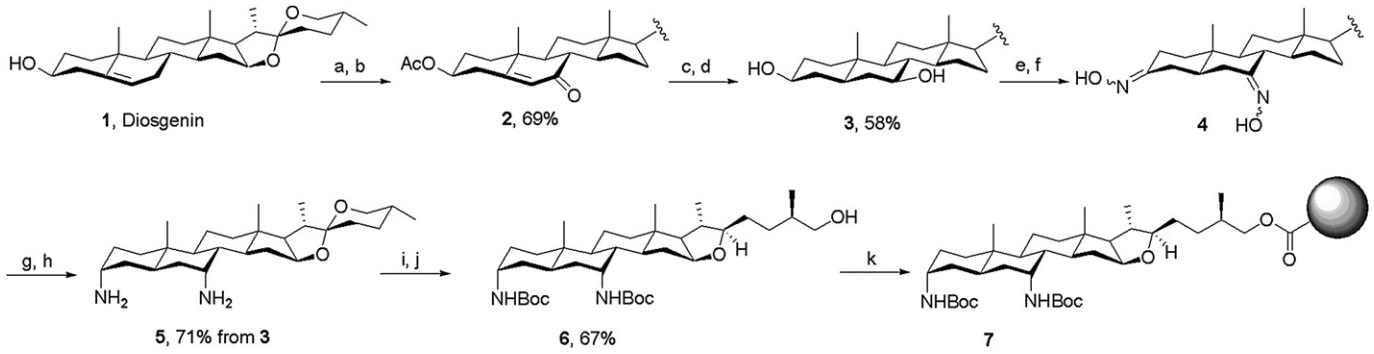
The use of furostanic sapogenins as organizing scaffolds for peptides or other recognition motifs has not been described so far. To our knowledge, only spirostanic sapogenins have been conjugated to (cyclo)peptidic moieties,[14] albeit this has been accomplished by Ugi-type multicomponent approaches that produce peptoids instead of peptides. The employment of the furostanic skeleton to prepare conjugates in which the peptide chains are aligned toward the α face shows promise in the fields of supramolecular and medicinal chemistry. Although we accept that this type of sapogenin skeleton cannot compete with the bile acids in terms of availability and low prize, it is indeed an interesting alternative to the use of the cholanic nucleus. For example, the former provides a novel topology based on the more extended and flatter shape of its polycyclic system, which is, e.g., more suitable for achieving facial amphiphilicity in steroidal conjugates. In addition, a variety of natural and synthetic furostanic derivatives have been found to be biologically active, as exemplified by the remarkable antitumoral activity of some carbohydrate–furostane conjugates (i.e., furostan saponins)[15] and the plant-growth promoting activity of various polyoxygenated furostanes analogous to the brassinosteroids.[16] This article focuses on illustrating the possibility of using diamino-furostanes as scaffolds for positioning peptides and cyclopeptides in an axially-disposed arrangement.

To accomplish our goal, the synthetic planning was directed toward the introduction of α-oriented amino groups at positions 3, 7, and 12 of furostanic skeletons. To ensure the axial disposition of the substituent at C-3, it was crucial either to employ sapogenins featuring a natural trans A/B-ring fusion or to implement approaches that provide this structural feature. This is important to impose conformational constrain to the conjugated peptide chains, as the axial substitution guarantees that the amide bond cannot rotate freely due to the 1,3-diaxial interactions. The preorganization achieved by this issue is known to be especially useful to enforce the peptide strands into specific types of folded motifs.

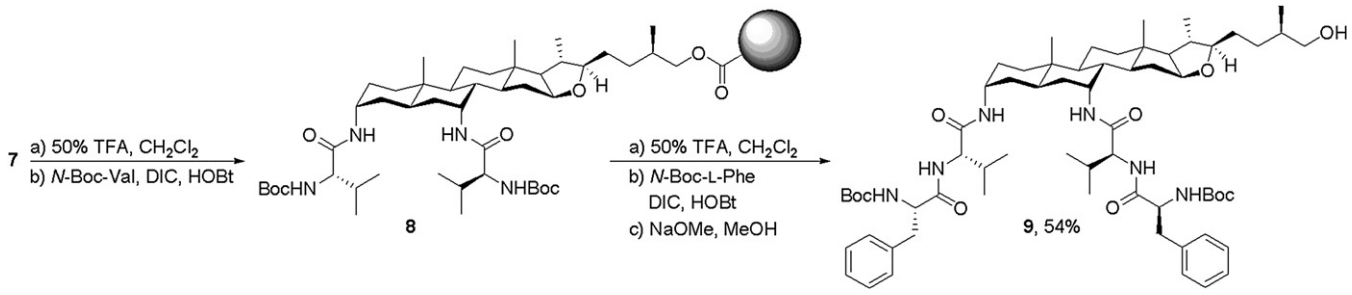
As depicted in Scheme 1, the synthetic route toward protected 3α,7α-diamino-furostane **6** commenced with functionalization of C-7 on the spirostan sapogenin diosgenin (**1**). This required the protection of the 3β-hydroxyl group and allylic oxidation of the Δ^5-olefin with *tert*-butyl hydroperoxide and copper iodide according to a reported procedure.[17] Further cleavage of the acetate and reduction of the α,β-unsaturated ketone furnished the 3β,7β-spirostanediol **3** in 58% yield after column chromatography. A minor amount of the untransformed enone was recovered from the column and used in further experiments. The reductive system Li/NH_3/*t*-BuOH was chosen to ensure the 5α-configuration of the resulting steroidal nucleus, as previously described in the literature.[10] Although several approaches are viable to prepare α-oriented amino A/B-trans steroids, oximation and reduction were chosen as it has previously proven successful in delivering the 3α,7α-diamino system in a reliable manner.[6,10] This procedure requires the use of the Adam's catalyst (i.e., platinum (IV) oxide hydrate) for the catalytic hydrogenation of the dioxime to the corresponding bis-hydroxylamine, which upon further reduction with zinc powder in acetic acid furnishes the desired stereochemical outcome. Accordingly, PCC oxidation of diol **3** to the corresponding diketone followed by oximation and reduction as described above afforded the 3α,7α-diamino-spirostane **5** in 71% yield. Minor amounts of the 3 and 7-epimers were detected, although difficult to isolate by column chromatography. The stereochemistry of the amino groups in **5** was confirmed by revealing the equatorial disposition of protons at C-3 and C-7 by ^1H NMR.

Once the 3α,7α-diamino system was introduced into the spirostanic skeleton, reductive opening of the spiroketal chain with $LiAlH_4/AlCl_3$ followed by amine protection with di-*tert*-butyl dicarbonate gave rise to the furostanic dicarbamate **6** in 67% yield. It must be noticed that the current reductive opening approach requires a larger amount of $AlCl_3$ than the one traditionally employed to reduce diosgenin to dihydrodiosgenin.[13e] This is a consequence of the presence of the amino groups, which inactivate a portion of the Lewis acid. Finally, compound **6** was coupled to acid chloride polystyrene resin[18] to give the solid-phase bound furostanic scaffold **7**. The remaining reactive sites of the resin were capped as methyl esters.[18]

It has been reported that the reductive opening of the spiroketal chain proceeds with complete retention of the configuration at C-22.[13,16] This has been proved for the two most commonly used protocols for producing furostanic derivatives, i.e., reduction with



Scheme 1. Synthesis of 3α,7α-diamino-furostanes. (a) Ac_2O, Et_3N, DMAP, CH_2Cl_2; (b) TBHP, CuI, TBAB, CH_2Cl_2–H_2O; (c) NaOH, MeOH; (d) Li, NH_3, THF, *t*-BuOH; (e) PCC, CH_2Cl_2; (f) $NH_2OH{\cdot}HCl$, NaOAc, MeOH, reflux; (g) H_2, PtO_2, HOAc; (h) Zn, AcOH, then aq $NaHCO_3$; (i) $LiAlH_4/AlCl_3$, THF, 0 °C→reflux; (j) Boc_2O, $NaHCO_3$, THF–H_2O; (k) acid chloride polystyrene resin, DMAP, CH_2Cl_2.



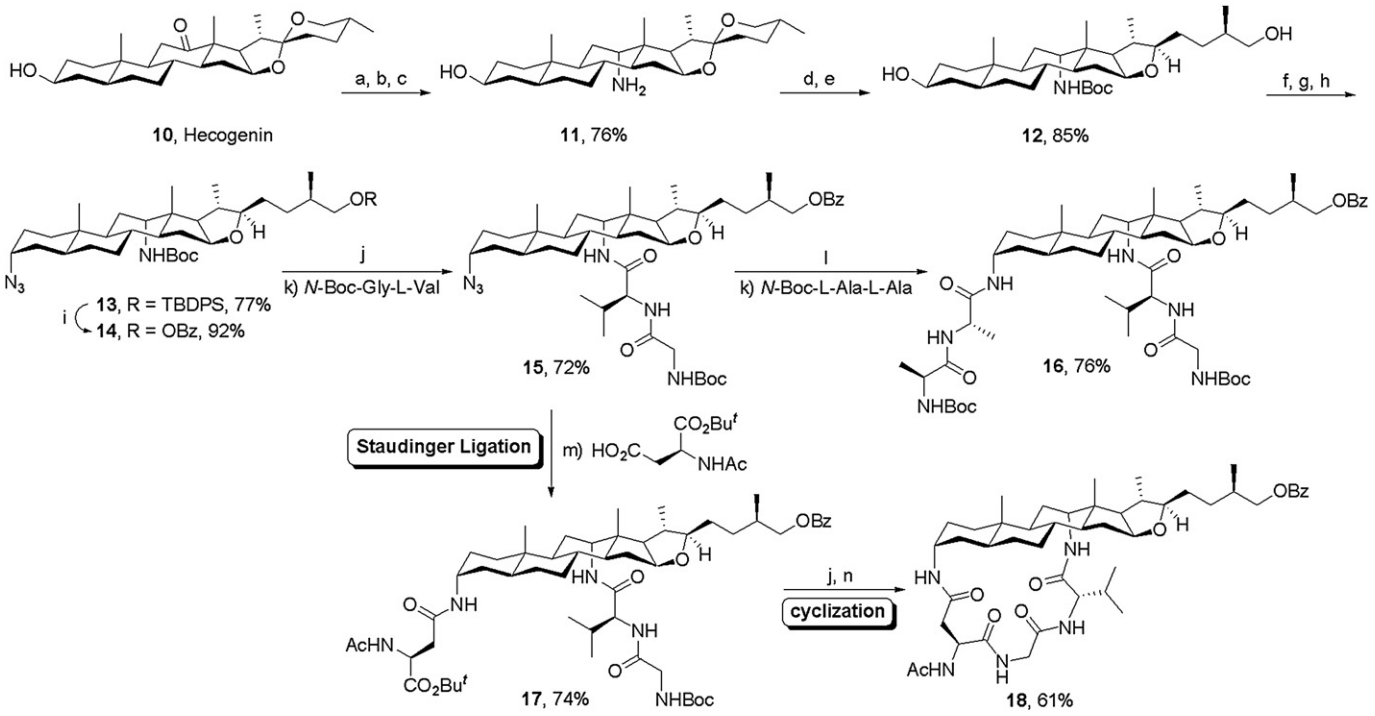
Scheme 2. Solid-phase synthesis of a peptide–furostane conjugate.

$AlCl_3$–$LiAlH_4$ and PtO_2-catalyzed hydrogenation under acidic conditions.[13b,e,16] The NMR data of the furostanes herein obtained are in agreement with that previously reported, thus confirming the *R* configuration at C-22 (β-orientation of the side chain). In our hands, the α-orientation of H-22 was confirmed through NOE difference experiments (irradiation of methyl-21) that showed the *syn* disposition between H-22 and methyl-21. This fact corroborates that the stereochemical outcome of the reductive opening procedure ($AlCl_3$–$LiAlH_4$) is independent on the functionalities at the steroidal nucleus; even if amino groups, that are likely to coordinate the Lewis acid, are nearby (ring C in compound **11**) the spiroketal chain.

The solid-phase assembly of both dipeptide chains at positions 3 and 7 was accomplished by using Boc-protected α-amino acids and the DIC/HOBt methodology. This procedure involved the successive incorporation of valine and phenylalanine to yield the peptidic arms aligned by the furostanic skeleton. As previously demonstrated with other steroidal scaffolds, larger peptide fragments can be grown by this standard methodology with the focus posed on specific applications (e.g., enzyme mimicry). Final release from the solid support provided the peptidofurostane **9** in 54% yield (based on **6**) after column chromatography (Scheme 2).

Besides the high availability of spirostan sapogenins, this family of natural products shares with the bile acids the advantage of appearing in nature functionalized at various positions. Accordingly, we turned to look for other fuctionalization patterns, thus focusing on the synthesis of a 3α,12α-diamino-5α-furostanic scaffold from a natural spirostan sapogenin. For this, the highly available hecogenin (**10**) was chosen as it already possesses the 5α-configuration and is naturally functionalized at C-3 and C-12. However, the goal of this next approach is not only to introduce the amino groups, but also to provide differentiation between the two positions for assembling dissimilar peptide chains via solution-phase synthesis.

Scheme 3 shows the preparation of the bifunctionalized furostanic scaffold and the stepwise synthesis of two dipeptide fragments at positions 3 and 12. This process required the individual incorporation of each amino group and the use of orthogonal protection in a protocol similar to other ones previously employed for cholanic substrates.[6] Thus, hecogenin (**10**) was subjected to oximation, followed by PtO_2-catalyzed hydrogenation and subsequent treatment with Zn/AcOH to complete the reduction to the amine. The amino-sapogenin **11** was obtained in 76% yield after basification and purification by column chromatography. Reductive opening of the spiroketal chain as described above followed by



Scheme 3. Synthesis of (cylco)peptide–furostane conjugates from 3α,12α-diamino-furostanes. (a) $NH_2OH{\cdot}HCl$, NaOAc, MeOH, reflux; (b) H_2, PtO_2, HOAc; (c) Zn, AcOH, then aq $NaHCO_3$; (d) $LiAlH_4/AlCl_3$, THF, 0 °C→reflux; (e) Boc_2O, $NaHCO_3$, THF–H_2O; (f) TBDPSCl, imidazole, CH_2Cl_2, 0 °C; (g) MsCl, Et_3N, CH_2Cl_2, 0 °C; (h) NaN_3, DMPU, 40 °C; (i) TBAF, THF, then BzCl, Py; (j) 40% TFA, CH_2Cl_2; (k) EDC, HOBt, DIPEA, CH_2Cl_2–DMF; (l) Me_3P, THF, then H_2O; (m) DCC, HOBt, CH_3CN, then Bu_3P; (n) HATU, 2,6-collidine, CH_2Cl_2.

amine protection as *tert*-butyl carbamate furnished compound **12** in 85% yield. To accomplish the selective incorporation of an amino group at C-3 without affecting the functionalized furostanic side chain, we turned to implement a selective protection procedure for the primary hydroxy group at C-26. This was accomplished by employing TBDPSCl under standard silylation conditions, albeit utilizing low temperature and a very short reaction time. Incorporation of the axial 3α-azido group was completed in overall 77% yield by mesylation of the equatorial 3β-OH followed by nucleophilic displacement with sodium azide. Finally, the TBDPS group was changed by benzoyloxy using standard deprotection/protection techniques, as the former protecting group showed to be partially cleaved during the peptide coupling sequence.

The differentially functionalized scaffold **14** proved to be suitable for the introduction of two different peptide fragments in a sequential manner. The peptidofurostane **15** was firstly produced in 72% yield by deprotection of the 12α-amino group followed by solution-phase coupling with EDC/HOBt-activated *N*-Boc–Gly–L-Val. Introduction of the second peptide chain (i.e., *N*-Boc–L-Ala–L-Ala) was accomplished by Staudinger reduction of the azido group followed by peptide coupling as described before to afford the conjugate **16** in overall 76% yield. The success of this route demonstrates that furostanic scaffolds can be readily functionalized in a differential manner for positioning not only dissimilar peptidic arms, but also other recognition and catalytic motifs. As it might be noticed, the possibility of locating different peptides into a furostanic skeleton is independent of the use of solid or solution-phase peptide synthesis.

Having established the methodology for incorporating amino acids and peptides into furostanic scaffolds, we envisaged the application of these latter also to impose conformational preorganization into cyclopeptide derivatives. Macrocyclic peptides comprise one of the most important classes of target compounds for drug discovery and biological chemistry.[19] Not only macrocycles composed of oligomeric sequences (e.g., cyclopeptides and depsipeptides), but also especially other types of biologically active, amino acid-derived macrocycles are of utmost importance as they are usually considered as lead compounds in medicinal chemistry.[19] The latter are featured by natural or synthetic hybrid skeletons that include rigid (aromatic) endocyclic motifs alternating with short peptide sequences.[19] Combined with cyclization, these characteristics allow introducing conformational restrictions into specific peptide sequences of biological relevance (e.g., RGD-containing peptides).[20,21] Cyclization is also widely employed with the aim to improve pharmacological properties (e.g., bioavailability and enzymatic stability)[22] and to decrease the entropy loss upon binding.[23] Encouraged by the great applicability of cyclopeptides and analogous macrocycles composed of peptidic hybrid scaffolds, we focused on addressing the potential of the extended and rigid furostanic skeleton for positioning a macrocyclic tripeptide in a preorganized form.

As shown in Scheme 3, the preparation of the cyclopeptide–furostane conjugate **18** was conducted by firstly incorporating an asparagine moiety at C-3 in conjugate **15**, followed by deprotection of the N- and C-terminus and consecutive cyclization by a standard ring-closing coupling protocol. For the incorporation of the side chain carboxylate of aspartic acid, an efficient, recently reported procedure based on the Staudinger methodology was employed.[24] This method was originally developed for the convergent synthesis of glycopeptides and shows promise for the selective ligation of azido building blocks to carboxylate-functionalized peptides. In our hands, it also proved to be suitable for the orthogonal incorporation of the asparagine moiety into the furostanic skeletons. The approach comprises the nucleophilic attack of the aza-ylide intermediate (generated by reaction of the steroidal azide with tributylphosphine) to *N*-protected L-aspartic acid α-*tert*-butyl ester suitably activated (DCC/HOBt) at the side chain carboxylate. Despite the axial character of the azido group, the reaction proceeded readily to produce the conjugate **17** in 74% yield and without affecting the carbamate and ester functionalities.

For the cyclization step, removal of the Boc group and the *tert*-butyl ester at the N- and C-terminus, respectively, of the hybrid peptide–steroid skeleton was previously required. This was accomplished in one step by treatment with TFA followed by conversion to the corresponding hydrochloride salt (HCl–EtOAc). This latter intermediate was submitted as crude product to cyclization by treatment with HATU in CH_2Cl_2 and employing 2,6-collidine as base. Thus, the ring closure proceeded smoothly within 36 h to afford the cyclopeptide–furostane conjugate **18** in 61% yield and without substantial racemization. Interestingly, neither larger excess of coupling reagents nor longer reaction times improved significantly the cyclization yield. Other peptide coupling reagents amenable for cyclization, such as: PyBroP (3 equiv, 6 equiv of DIPEA, CH_2Cl_2), BOP–Cl (3 equiv, 6 equiv of DMAP, CH_3CN), and EDC–HOAt (3 equiv, 6 equiv of $NaHCO_3$, DMF), were also probed for this goal. However, none of them was found to be more successful than HATU in terms of efficiency and feasibility of the workup. Although not examined in detail, the choice of the solvent also influenced the cyclization result. For example, the use of CH_2Cl_2 as solvent in the cyclization with HATU was more effective than DMF ($NaHCO_3$) and CH_3CN. On the other hand, DMF was superior to CH_2Cl_2 or a mixture of CH_2Cl_2–DMF in the alternative EDC–HOAt protocol.

As mentioned before, both the incorporation of rigid endocyclic motifs and cyclization are common approaches to impose conformational constrains into oligopeptide sequences. As these features influence the overall conformation of the cavity, they can be used (together or separately) to achieve specific types of peptide folding (e.g., β-turn mimic)[25] or to reduce the conformational flexibility of peptide fragments that are known to be recognized by biological receptors.[20–22] With the synthesis of macrocycle **18**, we illustrate the possibility of using steroidal sapogenins to restrict conformationally short peptide sequences. It is well-known that the recognition of various peptides and proteins is highly influenced by the 'active conformation' of specific sequences, which can be as short as 3 amino acid residues.[20,21] Therefore, we believe that the use of the rigid and extended furostanic skeleton can be extended to pursue the active conformation of biologically relevant peptide fragments. As previously shown, either 3,7- or 3,12-diamino-furostanes can be readily used as scaffolds for positioning peptidic arms. The same applies for attaching cyclopeptides to A/B- and A/C-bifunctional furostanic scaffolds, thus offering a great prospect for varying the cavity size, shape, and conformational flexibility of cyclic oligopeptide sequences.

3. Conclusions

It has been shown that furostanic sapogenins are amenable scaffolds for positioning (cyclo)peptides in a preorganized form. The approach comprises the introduction of α-oriented amino groups into spirostanic sapogenins and the subsequent reductive opening of the spiroketal chain to afford diamino-furostanic skeletons. These latter were then coupled to short peptide sequences by both solid and solution-phase syntheses. The functionalization of the furostanic skeleton proved to be a tunable process either for growing simultaneously two peptide chains or for assembling different peptide fragments in a consecutive manner. The peptide–furostane conjugates thus produced feature a trans A/B-ring fusion and hold the peptidic arms in axial disposition. This characteristic provides a preorganized array of peptidic motifs with potential applicability in molecular recognition and catalysis. A cyclopeptide–furostane conjugate was also produced with the aim to

illustrate the potential of the furostanic skeleton to impose conformational restrictions into short cyclopeptide sequences.

4. Experimental

4.1. General

Melting points were determined on a Stuart Scientific apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 spectrometer at 399.94 MHz and 100.57 MHz, respectively. Chemical shifts (δ) are reported in parts per million relative to TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR). The high resolution ESI mass spectra were obtained from a Bruker Apex 70e Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an Infinity™ cell, a 7.0 T superconducting magnet. Reactions were monitored by thin-layer chromatography on pre-coated plates with silica gel (Merck) and spots were visualized with a 1% w/v spray of vanillin in perchloric acid and subsequent heating. The solid compounds were recrystallized from selected solvents for the melting point measurements. Flash column chromatography was performed on silica gel 60 (Merck, >230 mesh). DMF, CH_2Cl_2, and DIPEA were dried by distillation from CaH_2. Boc-amino acids, HOBt, and peptides were purchased from Fluka. The carboxypolystyrene resin was purchased from NovaBiochem.

*4.1.1. (25R)-3β-Acetoxy-spirost-5-en-7-one (**2**)*

A solution of diosgenin (**1**, 2.48 g, 6.0 mmol) in 150 mL of CH_2Cl_2 was treated with Et_3N (20 mL), Ac_2O (6 mL), and DMAP (72 mg, 0.3 mmol). The reaction mixture was stirred at room temperature for 8 h and then poured into 300 mL of cold water and extracted with CH_2Cl_2 (2×100 mL). The organic layer was washed with aq 2 N HCl (2×80 mL) and brine (100 mL), dried over anhyd Na_2SO_4, and concentrated under reduced pressure to dryness. The resulting crude product was dissolved in 40 mL of CH_2Cl_2 and treated with CuI (775 mg, 4.0 mmol) and tetra-*n*-butyl ammonium bromide (232 mg, 0.72 mmol). *tert*-Butyl hydroperoxide (70% in H_2O, 5.7 mL, 60 mmol) was added and the reaction mixture was stirred at reflux. Further TBHP (5.7 mL, 60 mmol) was added after 1.5 h and 3 h. The reaction mixture was refluxed for additional 2 h, then poured into 100 mL of cold water and extracted with CH_2Cl_2 (2×100 mL). The organic layer was washed sequentially with aq 2 N HCl (50 mL), aq 10% $NaHSO_3$ (2×50 mL), and brine (50 mL), dried over anhyd Na_2SO_4, and concentrated under reduced pressure to dryness. Flash column chromatography purification (*n*-hexane/EtOAc 8:1) furnished the α,β-unsaturated ketone **2** (1.95 g, 69%) as a white solid. Diosgenin acetate (300 mg, 11%) was recovered from the column. Mp (from *n*-hexane/CH_2Cl_2): 194–195 °C. ^{1}H NMR ($CDCl_3$): δ=0.79 (d, 3H, *J*=6.2 Hz, H-27); 0.82 (s, 3H, H-18); 0.97 (d, 3H, *J*=6.2 Hz, H-21); 1.21 (s, 3H, H-19); 2.06 (s, 3H, *CH*$_3$CO); 3.37 (t, 1H, *J*=10.6 Hz, H-26ax); 3.45 (dd, 1H, *J*=4.1/10.8 Hz, H-26eq); 4.44 (m, 1H, H-16α); 4.72 (br m, 1H, H-3α); 5.72 (d, 1H, *J*=1.5 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ=14.5, 15.9, 16.5, 17.1, 20.9, 207.9, 21.4, 24.7, 28.7, 30.2, 31.3, 31.4, 35.5, 35.6, 39.8, 40.4, 40.5, 41.6, 41.8, 51.3, 55.4, 62.0, 66.8, 72.5, 80.6, 109.2, 128.5, 146.6, 170.1. HRMS (ESI-FT-ICR) *m*/*z*: 493.2937 $[M+Na]^+$; calcd for $C_{29}H_{42}NaO_5$: 493.2932.

*4.1.2. (25R)-5α-Spirostane-3β,7β-diol (**3**)*

Enone **2** (1.8 g, 3.8 mmol) was dissolved in a 5% solution of NaOH in MeOH (100 mL). The reaction mixture was stirred at 40 °C for 1 h, then concentrated under reduced pressure to half the volume and acidified with aq 2 N HCl to pH 3. The aqueous suspension was extracted with Et_2O (2×100 mL), and the combined organic extracts were dried over anhyd Na_2SO_4 and concentrated under reduced pressure to dryness. The resulting crude product was suspended in a mixture of 30 mL of dry THF and 3 mL of *t*-BuOH. The suspension was added dropwise to a freshly prepared solution of lithium wire (620 mg, 88 mmol) in 150 mL of liquid ammonia maintained at −20 °C. The reaction mixture was stirred for 1 h at −20 °C and then quenched by addition of NH_4Cl in excess and vigorous stirring. After evaporation of the ammonia, the mixture was poured into water and extracted with EtOAc (2×200 mL). The combined organic phase was washed with satd aq NH_4Cl, dried over anhyd Na_2SO_4, and concentrated under reduced pressure to dryness. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 20:1) to afford the diol **3** (953 mg, 58%) as a white solid. Mp (from EtOAc): 244–246 °C. ^{1}H NMR ($CDCl_3$): δ=0.79 (d, 3H, *J*=6.2 Hz, H-27); 0.81 (s, 3H, H-18); 0.97 (d, 3H, *J*=6.3 Hz, H-21); 1.08 (s, 3H, H-19); 3.36 (t, 1H, *J*=10.8 Hz, H-26ax); 3.47 (dd, 1H, *J*=10.9/4.0 Hz, H-26eq); 3.64 (m, 1H, H-3α); 3.71 (td, 1H, *J*=10.6/4.2 Hz, H-7α); 4.43 (m, 1H, H-16α). ^{13}C NMR ($CDCl_3$): δ=14.5, 15.6, 16.6, 17.1, 21.0, 28.8, 30.0, 30.1, 31.2, 31.4, 31.7, 35.0, 35.6, 38.6, 39.6, 40.1, 40.7, 41.7, 47.6, 54.4, 56.0, 62.2, 66.8, 71.4, 71.6, 81.0, 109.4. HRMS (ESI-FT-ICR) *m*/*z*: 433.3319 $[M+H]^+$; calcd for $C_{27}H_{45}O_4$: 433.3316.

*4.1.3. (25R)-3α,7α-Diamino-5α-spirostane (**5**)*

A solution of diol **3** (2.0 g, 4.7 mmol) in 100 mL of CH_2Cl_2 was added to a stirred suspension of pyridinium chlorochromate (PCC, 1.5 g, 7.0 mmol) in 60 mL of CH_2Cl_2 at 0 °C. The reaction mixture was stirred at room temperature for 5 h and then filtered through a pad of alumina. The solution was evaporated under reduced pressure to dryness and the resulting crude diketone was dissolved in 100 mL of MeOH. Sodium acetate (1.91 g, 23.3 mmol) and hydroxylamine hydrochloride (0.97, 14.0 mmol) were added and the reaction mixture was stirred at reflux for 8 h. The resulting suspension was cooled to room temperature, poured into 200 mL of cold water, and the precipitate was filtered under reduced pressure to afford the dioxime **4**. This product was dried at 60 °C and dissolved in glacial HOAc (40 mL). Platinum (IV) oxide hydrate (Adams's catalyst, 200 mg) was added and the reaction mixture was treated successively with vacuum and hydrogen and finally stirred under hydrogen atmosphere for 6 days. The suspension was filtered under reduced pressure and the catalyst was washed with glacial HOAc (2×20 mL). Zinc powder was added to the combined filtrates and the suspension was stirred at room temperature for 24 h and filtered to remove the zinc. The solvent was evaporated under reduced pressure and the resulting crude product was dissolved in 200 mL CH_2Cl_2. The organic phase was washed with satd aq $NaHCO_3$ (2×80 mL), dried over anhyd Na_2SO_4, and concentrated under reduced pressure to dryness. Flash column chromatography purification (*n*-hexane/EtOAc/Et_3N 3:1:0.1) yielded the pure diamine **5** (1.43 g, 71%) as a white foam. Mp (from MeOH): 221–223 °C. ^{1}H NMR ($CDCl_3$): δ=0.78 (d, 3H, *J*=6.5 Hz, H-27); 0.79 (s, 3H, H-18); 0.94 (s, 3H, H-19); 1.08 (d, 3H, *J*=6.4 Hz, H-21); 3.04 (br s, 1H, H-7β); 3.18 (br s, 1H, H-3β); 3.36 (t, 1H, *J*=11.0 Hz, H-26ax); 3.47 (dd, 1H, *J*=10.9/4.0 Hz, H-26eq); 4.43 (m, 1H, H-16α). ^{13}C NMR ($CDCl_3$): δ=11.6, 12.1, 14.6, 17.3, 27.2, 27.7, 29.2, 29.8, 30.4, 31.1, 31.3, 31.7, 32.4, 33.3, 34.2, 36.2, 40.0, 41.7, 47.4, 47.9, 51.2, 52.8, 60.6, 61.2, 62.1, 66.6, 80.1, 109.3. HRMS (ESI-FT-ICR) *m*/*z*: 431.3634 $[M+H]^+$; calcd for $C_{27}H_{47}O_2N_2$: 431.3639.

*4.1.4. (25R)-3α,7α-Di-[N-(tert-butoxycarbonyl)amino]-5α-furostan-26-ol (**6**)*

$LiAlH_4$ (1.44 g, 37.5 mmol) was carefully added to a stirred suspension of $AlCl_3$ (21.0 g, 0.15 mol) in dry Et_2O (150 mL) at 0 °C. A solution of amine **5** (1.08 g, 2.5 mmol) in dry Et_2O (50 mL) was added dropwise over a period of 15 min. Stirring was continued for 15 min at 0 °C, then for 1 h at room temperature and finally for 1 h at reflux. The mixture was treated cautiously with aq 10% NaOH (80 mL) and the resulting white powder was filtered off and washed several times with EtOAc. The combined organic phase was

washed with aq 10% $NaHCO_3$ and concentrated under reduced pressure to dryness. The resulting crude product was dissolved in a stirred mixture of THF (60 mL) and satd aq $NaHCO_3$ (30 mL) and treated with di-*tert*-butyl dicarbonate (Boc_2O, 1.3 g, 7.0 mmol). The reaction mixture was stirred for 30 h at room temperature, and then the layers were separated and the aqueous phase was extracted with EtOAc (2×100 mL). The organic extracts were combined, washed with brine (50 mL), dried over anhyd Na_2SO_4, and evaporated under reduced pressure to dryness. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) afforded the dicarbamate **6** (1.06 g, 67%) as a white powder. Mp (from *n*-hexane/CH_2Cl_2): 227–230 °C. ^{1}H NMR ($CDCl_3$): δ=0.80 (s, 3H, H-18); 0.84 (s, 3H, H-19); 0.92 (d, 3H, *J*=6.6 Hz, H-27); 0.98 (d, 3H, *J*=6.8 Hz, H-21); 1.44 (s, 9H, (CH_3)$_3$C); 1.45 (s, 9H, (CH_3)$_3$C); 3.32 (m, 1H, H-22α); 3.44–3.52 (m, 2H, H-26); 3.73 (m, 1H, H-7β); 3.92 (m, 1H, H-3β); 4.31 (br m, 1H, H-16α); 5.23 (br s, 1H, N*H*); 5.36 (br s, 1H, N*H*). ^{13}C NMR ($CDCl_3$): δ=16.4, 16.5, 18.3, 18.8, 20.6, 28.3, 28.45, 28.5, 28.6, 30.2, 30.4, 31.5, 32.0, 32.2, 35.7, 37.0, 38.1, 39.1, 39.4, 42.2, 46.8, 47.9, 50.0, 54.5, 56.7, 56.5, 67.8, 78.8, 78.9, 83.3, 90.4, 155.4, 155.5. HRMS (ESI-FT-ICR) *m*/*z*: 655.4770 [M+Na]$^+$; calcd for $C_{37}H_{64}N_2NaO_6$: 655.4765.

4.1.5. Preparation of the resin-bound furostanic dicarbamate **7**

Acid polystyrene resin (1.05 g, theoretical loading of 1.18 mmol of acid/g), 2.10 mL of freshly distilled thionyl chloride and 20 mL of dry benzene were stirred at reflux under nitrogen atmosphere for 12 h. The beads were filtered, washed with dry benzene (20 mL), then with dry Et_2O (2×20 mL), and dried under high vacuum. Furostanol **6** (520 mg, 0.82 mmol) was shaken with the acid chloride resin (695 mg), DMAP (10.0 mg, 0.082 mmol), and Et_3N (0.11 mL, 0.82 mmol) in dry DMF (5 mL) for 6 h. The beads were filtered and washed subsequently with DMF (3×30 mL), MeOH (3×30 mL), and CH_2Cl_2 (3×30 mL). In order to cap the reacting sites, the resin was shaken for 3 h in a solution of CH_2N_2 (2.5 mmol) in 20 mL of Et_2O, then filtered and washed subsequently with DMF (3×30 mL), MeOH (3×30 mL), and CH_2Cl_2 (3×30 mL) and dried in vacuum.

4.1.6. Synthesis of the peptide chains and cleavage to obtain the peptidofurostane **9**

The Boc-protecting groups on **7** were removed by adding 50% TFA in CH_2Cl_2 (30 mL) to the loaded beads (700 mg) and shaking the suspension for 2 h. The beads were subsequently washed with CH_2Cl_2 (3×30 mL), 10% DIPEA in DMF (3×30 mL), MeOH (3×30 mL), and CH_2Cl_2 (3×30 mL). *N*-Boc–L-valine (709 mg, 3.28 mmol, 4 equiv) was activated by treatment with HOBt (442 mg, 3.3 mmol) and DIC (0.52 mL, 3.28 mmol) in CH_2Cl_2–DMF (15 mL, 4:1, v/v) under nitrogen atmosphere for 1 h. The resulting solution was added to the above resin and the suspension was shaken for 12 h. The beads were subsequently washed with DMF (3×30 mL), MeOH (3×30 mL), and CH_2Cl_2 (3×30 mL). The coupling reaction was repeated twice to ensure complete coupling. Deprotection of the Boc groups on **8** and neutralization of the resin was accomplished as before and the beads were dried under vacuum. A small amount of the resin was separated and the furostanic scaffold was cleaved and analyzed by ESI-MS to monitor the coupling success. The subsequent coupling with 4-fold excess of activated (HOBt and DIC under nitrogen for 1 h) *N*-Boc–L-phenylalanine (709 mg, 3.28 mmol) was repeated twice as described before. Finally, the resin was washed subsequently with MeOH (3×30 mL) and CH_2Cl_2 (3×30 mL) and the beads were dried in vacuum. To cleave the peptidofurostane, the resin was shaken in 8 mL of 1 M solution of NaOMe in MeOH for 3 h. The beads were filtered and washed subsequently with DMF (2×30 mL), MeOH (2×30 mL), and CH_2Cl_2 (2×30 mL) and the combined filtrate was concentrated under reduced pressure to dryness. The crude product was purified by flash column chromatography purification (CH_2Cl_2/MeOH/Et_3N 25:1:0.1) to afford the peptidofurostane **9** (495 mg, 54%) as a white powder. Mp (from MeOH): 269–270 °C. ^{1}H NMR ($CDCl_3$): δ=0.83 (s, 3H, H-18); 0.90 (s, 3H, H-19); 0.91, 0.93, 0.95 (3×d, 9H, 2×C*H*$_3$+3H-27); 0.98 (d, 3H, *J*=6.8 Hz, H-21); 1.43 (s, 9H, (CH_3)$_3$C); 1.45 (s, 9H, (CH_3)$_3$C); 3.03–3.24 (m, 4H, 2×C*H*$_2$); 3.33 (m, 1H, H-22α); 3.46–3.52 (m, 2H, H-26); 3.98–4.51 (m, 7H, 4×C*H*+H-3β+H-12β+H-16α); 5.28 (br s, 1H, N*H*); 5.37 (br s, 1H, N*H*); 5.86 (br s, 1H, N*H*); 5.99 (br s, 1H, N*H*); 7.17–7.29 (m, 10H, Ph). ^{13}C NMR ($CDCl_3$): δ=16.4, 16.5, 17.4, 17.5, 18.3, 18.8, 19.1, 19.5, 20.6, 28.3, 28.45, 28.6, 30.3, 30.4, 31.4, 32.1, 32.3, 35.7, 36.2, 36.8, 37.1, 37.4, 38.3, 39.4, 42.3, 44.1, 44.5, 44.9, 45.3, 46.8, 50.0, 55.8, 56.5, 56.9, 64.8, 67.9, 79.2, 79.6, 83.3, 90.3, 126.8, 127.0, 128.4, 128.5, 129.1, 135.7, 136.1, 136.3, 136.5, 156.9, 156.6, 171.0, 171.4, 172.8, 173.2. HRMS (ESI-FT-ICR) *m*/*z*: 1147.7437 [M+Na]$^+$; calcd for $C_{65}H_{100}N_6NaO_{10}$: 1147.7400.

4.1.7. (25R)-12α-Amino-5α-spirostan-3β-ol (**11**)

Hecogenin (**10**, 2.0 g, 4.65 mmol) was subjected to oxime formation and PtO_2-catalyzed reduction in a similar way as described in Section 4.1.3. Flash column chromatography purification (CH_2Cl_2/MeOH/Et_3N 30:1:1) afforded the amine **11** (1.52 g, 76%) as a white foam. Mp (from EtOAc): 232–233 °C. ^{1}H NMR ($CDCl_3$): δ=0.76 (s, 3H, H-18); 1.17 (s, 3H, H-19); 0.78 (d, 3H, *J*=6.5 Hz, H-27); 0.94 (d, 3H, *J*=6.5 Hz, H-21); 3.36 (t, 1H, *J*=10.9 Hz, H-26ax); 3.49 (dd, 1H, *J*=10.8/3.9 Hz, H-26eq); 3.56 (br m, 1H, H-3α); 3.19 (br s, 1H, H-12β); 4.40 (m, 1H, H-16α). ^{13}C NMR ($CDCl_3$): δ=11.9, 13.2, 16.1, 17.1, 27.2, 28.1, 28.8, 30.2, 31.2, 31.4, 33.8, 34.3, 36.1, 36.2, 37.7, 42.2, 44.4, 53.5, 55.0, 55.4, 55.6, 59.6, 61.2, 66.8, 71.1, 79.2, 109.3. HRMS (ESI-FT-ICR) *m*/*z*: 432.3479 [M+H]$^+$; calcd for $C_{27}H_{46}NO_3$: 432.3476.

4.1.8. (25R)-12α-[N-(tert-Butoxycarbonyl)amino]-5α-furostane-3β,26-diol (**12**)

Amine **11** (1.08 g, 2.5 mmol) was subjected to reductive opening of the spiroketal chain followed by amine protection with Boc_2O in a similar way as described in Section 4.1.4. Flash column chromatography purification (*n*-hexane/EtOAc 2:1) afforded the carbamate **12** (1.01 g, 76%) as a white powder. Mp (from *n*-hexane/CH_2Cl_2): 240–241 °C. ^{1}H NMR ($CDCl_3$): δ=0.80 (s, 3H, H-18); 0.84 (s, 3H, H-19); 0.92 (d, 3H, *J*=6.7 Hz, H-27); 0.97 (d, 3H, *J*=6.8 Hz, H-21); 1.45 (s, 9H, (CH_3)$_3$C); 3.31 (m, 1H, H-22α); 3.53 (br m, 1H, H-3α); 3.44–3.52 (m, 2H, H-26); 3.98 (br s, 1H, H-12β); 4.31 (br m, 1H, H-16α); 5.35 (br s, 1H, N*H*). ^{13}C NMR ($CDCl_3$): δ=16.4, 16.5, 18.3, 18.8, 20.6, 28.4, 28.5, 30.2, 30.4, 31.5, 32.0, 32.2, 35.7, 37.0, 37.2, 38.1, 39.4, 42.2, 46.8, 50.0, 54.1, 56.7, 56.5, 64.8, 67.9, 79.2, 83.3, 90.4, 155.4. HRMS (ESI-FT-ICR) *m*/*z*: 556.3981 [M+Na]$^+$; calcd for $C_{32}H_{55}NNaO_5$: 556.3976.

4.1.9. (25R)-3α-Azido-26-(tert-butyldiphenylsilyloxy)-12α-[N-(tert-butoxycarbonyl)amino]-5α-furostane (**13**)

TBDPSCl (0.5 mL, 1.98 mmol) was added dropwise to a stirred mixture of diol **12** (880 mg, 1.65 mmol), imidazole (275 mg, 1.8 mmol), and DMAP (catalytic amount) in CH_2Cl_2 (50 mL) at 0 °C. The mixture was allowed to reach room temperature, stirred for 30 min, and then diluted with 200 mL of CH_2Cl_2. The organic phase was washed with aq 10% HCl (2×50 mL), aq 10% $NaHCO_3$ (50 mL), and brine (50 mL), dried over anhyd Na_2SO_4, and evaporated under reduced pressure. The crude produce was dissolved in dry CH_2Cl_2 (80 mL) and treated with Et_3N (1.0 mL, 7.4 mmol), and mesyl chloride (0.29 mL, 2.45 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then diluted with 100 mL of CH_2Cl_2 and washed with brine (2×50 mL). The organic phase was dried over anhyd Na_2SO_4 and concentrated under reduce pressure to dryness. The resulting crude product was dissolved in DMPU (50 mL) and the solution was treated with NaN_3 (185 mg, 3.3 mmol). The reaction mixture was stirred vigorously under nitrogen atmosphere at 50 °C for 24 h and then diluted with 200 mL of Et_2O. The organic

phase was washed with aq 10% HCl (2×60 mL) and brine (100 mL), dried over anhyd Na_2SO_4, and evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 5:1) to give the pure azide **13** (1.01 g, 77%) as a colorless syrup. ^{1}H NMR ($CDCl_3$): δ=0.81 (s, 3H, H-18); 0.82 (s, 3H, H-19); 0.91 (d, 3H, J=6.8 Hz, H-27); 0.97 (d, 3H, J=6.8 Hz, H-21); 1.00 (s, 9H, $(CH_3)_3CSi$); 1.43 (s, 9H, $(CH_3)_3C$); 3.33 (m, 1H, H-22α); 3.47–3.54 (m, 2H, H-26); 3.67 (m, 1H, H-3β); 3.98 (br s, 1H, H-12β); 4.32 (br m, 1H, H-16α); 5.36 (br s, 1H, N*H*); 7.62–7.59 (m, 4H, Ar); 7.47–7.42 (m, 6H, Ar). ^{13}C NMR ($CDCl_3$): δ=16.4, 16.6, 18.4, 18.9, 20.8, 26.8, 28.45, 28.5, 30.1, 30.3, 31.5, 32.0, 32.4, 35.9, 37.2, 37.4, 37.9, 40.8, 42.4, 45.4, 50.1, 53.8, 56.7, 58.9, 64.8, 68.5, 78.3, 83.2, 90.3, 127.8, 129.7, 132.9, 135.3, 155.4. HRMS (ESI-FT-ICR) m/z: 819.5229 $[M+Na]^+$; calcd for $C_{48}H_{72}N_4NaO_4Si$: 819.5224.

4.1.10. *(25R)-3α-Azido-12α-[N-(tert-butoxycarbonyl)amino]-5α-furostan-26-yl benzoate (**14**)*

Tetra-*n*-butylammonium fluoride trihydrate (0.59 g, 1.9 mmol) was added to a solution of compound **13** (1.0 g, 1.25 mmol) in THF (30 mL) and the reaction mixture was stirred for 12 h. The solution was diluted with 100 mL of Et_2O, washed with aq 10% $NaHCO_3$ (2×50 mL), and brine (50 mL), dried over anhyd Na_2SO_4, and evaporated under reduced pressure to dryness. The crude product was dissolved in 50 mL of dry pyridine and treated with benzoyl chloride (215 mg, 1.5 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 5 h and then poured into 100 mL of water. The precipitate was filtered under reduced pressure, washed several times with water and dried at 60 °C to afford the benzoate **14** (761 mg, 92%) as a crude product. A small sample was recrystallized from EtOH–H_2O (4:1, v/v) for characterization. Mp: 233–234 °C. ^{1}H NMR ($CDCl_3$): δ=0.80 (s, 3H, H-18); 0.83 (s, 3H, H-19); 0.91 (d, 3H, J=6.6 Hz, H-27); 0.95 (d, 3H, J=6.7 Hz, H-21); 3.32 (m, 1H, H-22α); 3.68 (m, 1H, H-3β); 3.97 (br s, 1H, H-12β); 4.29–4.40 (m, 3H, H-16α+2H-26); 5.39 (br s, 1H, N*H*); 7.39–7.74 (m, 5H, Bz). ^{13}C NMR ($CDCl_3$): δ=16.4, 16.6, 18.4, 20.3, 28.1, 28.3, 28.4, 30.1, 30.4, 31.6, 32.1, 32.3, 36.1, 37.2, 37.8, 38.3, 39.7, 40.2, 42.3, 44.7, 50.3, 53.4, 55.6, 58.8, 66.3, 70.4, 78.2, 83.1, 90.2, 125.4, 128.7, 129.5, 130.2, 130.5, 133.4, 155.4, 168.4. HRMS (ESI-FT-ICR) m/z: 685.4350 $[M+Na]^+$; calcd for $C_{39}H_{58}N_4NaO_5$: 685.4306.

4.1.11. *Peptidofurostane **15***

Compound **14** (662 mg, 1.0 mmol) was dissolved in CH_2Cl_2 (15 mL) and treated with trifluoracetic acid (TFA, 10 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 2 h. The solvent was evaporated under reduced pressure and the resulting product was dissolved in CH_2Cl_2 (50 mL), washed with satd aq Na_2CO_3 (2×10 mL), and dried over anhyd Na_2SO_4. Evaporation under reduced pressure gave the free amine as pale yellow foam. This compound was added to a solution of *N*-Boc–Gly–L-Val (411 mg, 1.5 mmol) and DIPEA (0.2 mL, 1.2 mmol) in CH_2Cl_2–DMF (10 mL, 4:1, v/v). The mixture was treated with EDC (286 mg, 1.5 mmol) and HOBt (203 mg, 1.5 mmol) and stirred at room temperature under nitrogen atmosphere for 24 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with aq 10% $NaHCO_3$ (2×50 mL), aq 10% HCl (50 mL), and brine (50 mL), and then dried over anhyd Na_2SO_4 and evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 15:1) to give the conjugate **15** (589 mg, 72%) as a white solid. Mp (from acetone): 238–239 °C. ^{1}H NMR ($CDCl_3$): δ=0.80 (s, 3H, H-18); 0.84 (s, 3H, H-19); 0.88, 0.90, 0.92 (3×d, 9H, 2×CH_3+3H-27); 0.96 (d, 3H, J=6.8 Hz, H-21); 1.44 (s, 9H, $(CH_3)_3C$); 3.31 (m, 1H, H-22α); 3.66 (m, 1H, H-3β); 3.79 (m, 1H); 4.00–4.58 (m, 6H); 4.99 (br s, 1H, N*H*); 5.19 (br s, 1H, N*H*); 7.34–7.71 (m, 5H, Bz). ^{13}C NMR ($CDCl_3$): δ=16.5, 16.8, 17.5, 18.3, 18.6, 20.5, 28.3, 28.4, 28.5, 30.3, 30.4, 31.6, 32.0, 32.4, 35.7, 36.8, 37.2, 38.2, 39.1, 39.4, 42.2, 44.5, 46.4, 50.6, 55.9, 56.4, 64.8, 67.9, 77.8, 83.3, 90.2, 125.2, 128.6, 129.7, 130.5, 130.6, 133.7, 155.6, 167.1, 170.8, 172.6. HRMS (ESI-FT-ICR) m/z: 841.5210 $[M+Na]^+$; calcd for $C_{46}H_{70}N_6NaO_7$: 841.5206.

4.1.12. *Peptidofurostane **16***

Azide **15** (305 mg, 0.4 mmol) was dissolved in dry THF (5 mL) and treated with trimethylphosphine (1 M in THF, 0.48 mL, 0.48 mmol). The reaction mixture was stirred at room temperature for 5 h, then water (0.15 mL) was added and stirring was continued for 6 h. The volatiles were evaporated under reduced pressure and the crude product was dried by repeated addition of toluene and evaporation. The resulting amine was added to a solution of *N*-Boc–L-Ala–L-Ala (156 mg, 0.6 mmol) and DIPEA (0.1 mL, 0.6 mmol) in CH_2Cl_2–DMF (8 mL, 4:1, v/v). The mixture was treated with EDC (114 mg, 1.5 mmol) and HOBt (203 mg, 1.5 mmol) and stirred at room temperature under nitrogen atmosphere for 48 h. The mixture was diluted with CH_2Cl_2 (80 mL) and washed with aq 10% $NaHCO_3$ (2×20 mL), aq 10% HCl (20 mL), and brine (20 mL), and then dried over anhyd Na_2SO_4 and evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 12:1) to give the conjugate **16** (314 mg, 76%) as a white foam. Mp (from EtOAc): 247–248 °C. ^{1}H NMR ($CDCl_3$): δ=0.81 (s, 3H, CH_3); 0.83 (s, 3H, CH_3); 0.87–0.94 (9H, 3×CH_3); 0.96 (d, 3H, J=6.6 Hz, H-21); 1.44 (s, 9H, $(CH_3)_3C$); 1.46 (s, 9H, $(CH_3)_3C$); 1.53 (d, J=6.6 Hz, 3H, CH_3); 1.53 (d, J=7.2 Hz, CH_3); 1.56 (d, J=7.0 Hz, CH_3); 3.32 (m, 1H, H-22α); 4.00–4.59 (m, 10H, 3×CH+CH_2+H-3β+H-12β+2H-26+H-16α); 5.09 (br s, 1H, N*H*); 5.22 (br s, 1H, N*H*); 5.29 (br s, 1H, N*H*); 5.38 (br s, 1H, N*H*); 5.92 (br s, 1H, N*H*); 7.36–7.76 (m, 5H, Bz). ^{13}C NMR ($CDCl_3$): δ=16.5, 16.8, 17.5, 17.7, 17.9, 18.3, 18.6, 20.5, 28.3, 28.4, 28.5, 28.6, 30.2, 30.3, 31.5, 32.5, 32.8, 35.2, 37.1, 37.7, 38.4, 39.1, 40.8, 42.6, 44.1, 46.9, 48.9, 49.3, 50.2, 54.9, 62.8, 64.9, 67.9, 78.2, 78.4, 83.6, 90.3, 125.2, 128.5, 129.8, 130.3, 130.7, 133.5, 156.0, 156.3, 167.8, 170.3, 171.8, 173.3, 173.6. HRMS (ESI-FT-ICR) m/z: 1057.6564 $[M+Na]^+$; calcd for $C_{57}H_{90}N_6NaO_{11}$: 1057.6568.

4.1.13. *Peptidofurostane **17***

A solution of *N*-Ac-L-aspartic acid α-*tert*-butyl ester (76 mg, 0.33 mmol), HOBt (45 mg, 0.33 mmol), and DCC (68 mmol, 0.33 mmol) in dry CH_3CN was stirred under nitrogen atmosphere for 30 min. Azide **15** (250 mg, 0.33 mmol) and tributylphosphine (67 mg, 0.33 mmol) were then added and the reaction mixture was stirred for additional 24 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH 12:1) to afford the conjugate **17** (245 mg, 74%) as a white foam. Mp (from MeOH): 242–243 °C. ^{1}H NMR ($CDCl_3$): δ=0.79 (s, 3H, H-18); 0.83 (s, 3H, H-19); 0.87, 0.90, 0.91 (3×d, 9H, 2×CH_3+3H-27); 0.95 (d, 3H, J=6.7 Hz, H-21); 1.44 (s, 9H, $(CH_3)_3C$); 1.48 (s, 9H, $(CH_3)_3C$); 2.04 (s, 3H, Ac); 2.67–2.74 (m, 2H, CH_2); 3.31 (m, 1H, H-22α); 3.88 (m, 1H, H-3β); 3.96 (m, 1H); 4.06–4.62 (m, 7H); 5.17 (m, 1H, N*H*); 5.33 (m, 1H, N*H*); 5.47 (d, 1H, J=8.0 Hz, N*H*); 7.39–7.70 (m, 5H, Bz). ^{13}C NMR ($CDCl_3$): δ=16.9, 17.2, 17.5, 18.1, 18.5, 20.5, 20.9, 28.2, 28.3, 28.4, 28.45, 28.5, 30.1, 30.3, 31.5, 32.3, 32.6, 34.8, 35.5, 36.4, 37.1, 37.8, 38.8, 39.4, 39.8, 40.4, 42.3, 45.0, 46.2, 50.3, 51.3, 55.5, 67.9, 68.6, 70.3, 77.8, 82.8, 83.2, 90.3, 125.2, 128.7, 129.6, 130.5, 130.7, 133.6, 155.5, 168.0, 170.7, 170.9, 171.4, 172.6. HRMS (ESI-FT-ICR) m/z: 1028.6270 $[M+Na]^+$; calcd for $C_{56}H_{87}N_5NaO_{11}$: 1028.6294.

4.1.14. *Cyclopeptidofurostane **18***

TFA (10 mL) was added to a solution of compound **17** (220 mg, 0.22 mmol) in CH_2Cl_2 (15 mL), and the mixture was stirred at room temperature for 2 h. The volatiles were removed by evaporation, and the residue was treated subsequently with CH_2Cl_2 and concentrated under reduced pressure. The residue was then suspended in 10 mL of 3.5 M HCl–EtOAc, stirred for 5 min, and the solvent was evaporated under reduced pressure. The resulting crude product was dried, suspended in 50 mL of dry CH_2Cl, and then treated with 2,6-collidine (0.25 mL, 2.0 mmol) and HATU (250 mg, 0.66 mmol)

under nitrogen to afford a pale brown solution. The reaction mixture was stirred for 12 h at room temperature and additional HATU (125 mg, 0.33 mmol) was added. Stirring was continued for 24 h at room temperature, and the solvent was removed under reduced pressure. Flash column chromatography purification (CH_2Cl_2/MeOH 18:1) afforded the cyclopeptide–furostane conjugate **18** (111 mg, 61%) as a pale yellow solid. Mp (from acetone): 255–257 °C. ^{1}H NMR ($CDCl_3$): δ=0.80 (s, 3H); 0.82 (s, 3H); 0.88, 0.90, 0.92 (3×d, 9H, 2×C*H*$_3$+3H-27); 0.96 (d, 3H, *J*=6.8 Hz, H-21); 2.01 (s, 3H, Ac); 2.72 (dd, 1H, *J*=15.0/7.1 Hz); 2.78 (dd, 1H, *J*=14.9/7.2 Hz); 3.33 (m, 1H, H-22α); 3.90 (m, 1H); 3.98 (m, 1H); 4.22 (dd, 1H, *J*=14.3/6.8 Hz); 4.29 (dd, 1H, *J*=14.4/6.7 Hz); 4.31–4.58 (m, 6H); 5.54 (m, 1H, N*H*); 5.73 (m, 1H, N*H*); 6.12 (d, 1H, *J*=7.9 Hz, N*H*); 7.37–7.71 (m, 5H, Bz). ^{13}C NMR ($CDCl_3$): δ=16.8, 17.4, 17.8, 18.2, 18.6, 18.7, 20.8, 21.0, 30.3, 30.5, 31.8, 32.6, 32.9, 34.7, 35.5, 36.4, 37.1, 37.8, 38.8, 39.4, 39.8, 40.4, 42.3, 45.0, 46.2, 50.3, 51.6, 54.8, 67.7, 68.8, 70.2, 77.4, 90.3, 125.4, 128.8, 129.8, 130.4, 130.7, 133.5, 168.1, 169.7, 170.9, 171.2, 171.3, 172.4. HRMS (ESI-FT-ICR) *m*/*z*: 854.5036 [M+Na]$^+$; calcd for $C_{47}H_{69}N_5NaO_8$: 854.5040.

Acknowledgements

We are grateful to the Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany, for providing the HRMS measurements and for the generous gift of some chemicals. We also thank the Institute of Materials, Universidad Nacional Autónoma de México, for some NMR measurements.

References and notes

1. (a) Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4130–4163; (b) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3118–3130; (c) Wennemers, H.; Conza, M.; Nold, M.; Krattiger, P. *Chem.—Eur. J.* **2001**, *7*, 3342–3347; (d) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479–2494; (e) Schneider, H.-J. *Adv. Supramol. Chem.* **2000**, *6*, 185–216; (f) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155–163.
2. (a) Chamorro, C.; Liskamp, R. J. M. *Tetrahedron* **2004**, *60*, 11145–11157; (b) Chamorro, C.; Liskamp, R. J. M. *J. Comb. Chem.* **2003**, *5*, 794–801; (c) Rump, E. T.; Rijkers, D. T. S.; Hilbers, H. W.; de Groot, P. G.; Liskamp, R. J. M. *Chem.—Eur. J.* **2002**, *8*, 4613–4621; (d) Causton, A. S.; Sherman, J. C. *Bioorg. Med. Chem.* **1999**, *7*, 23–27.
3. (a) Castani, A.; Sansone, F.; Ungaro, R. *Acc. Chem. Res.* **2003**, *36*, 246–254; (b) Lazzarotto, M.; Sansone, F.; Baldini, L.; Casnati, A.; Cozzini, P.; Ungaro, R. *Eur. J. Org. Chem.* **2001**, 595–602; (c) Sansone, F.; Barboso, S.; Casnati, A.; Fabbi, M.; Pochini, A.; Ugozzoli, F.; Ungaro, R. *Eur. J. Org. Chem.* **1998**, 897–905; (d) Park, H. S.; Lin, Q.; Hamilton, A. D. *J. Am. Chem. Soc.* **1999**, *121*, 8–13; (e) Hamuro, Y.; Calama, M. C.; Park, H. S.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **1997**, *36*, 2680–2683.
4. (a) Wright, A. T.; Anslyn, E. V. *Chem. Soc. Rev.* **2006**, *35*, 14–28; (b) Wright, A. T.; Zhong, Z.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2005**, *44*, 5679–5682; (c) Wright, A. T.; Griffin, M. J.; Zhong, Z.; MacCleskey, S. C.; Anslyn, E. V.; McDevitt, J. T. *Angew. Chem., Int. Ed.* **2005**, *44*, 6375–6378.
5. (a) Kwak, J.; Jefferson, E. A.; Bhumralkar, M.; Goodman, M. *Bioorg. Med. Chem.* **1999**, *7*, 153–160; (b) Melacini, G.; Feng, Y.; Goodman, M. *J. Am. Chem. Soc.* **1996**, *118*, 10359–10364; (c) Goodman, M.; Feng, Y.; Melacini, G.; Taulane, J. P. *J. Am. Chem. Soc.* **1996**, *118*, 5156–5157.
6. (a) del Amo, V.; Siracusa, L.; Markidis, T.; Baragaña, B.; Bhattarai, K. M.; Galobardes, M.; Naredo, G.; Pérez-Payán, M. N.; Davis, A. P. *Org. Biol. Chem.* **2004**, *2*, 3320–3328; (b) Ding, B.; Taotofa, U.; Orsak, T.; Chadwell, M.; Savage, P. B. *Org. Lett.* **2004**, *6*, 3433–3436; (c) Madder, A.; Li, L.; De Muynck, H.; Farcy, N.; Van Haver, D.; Fant, F.; Vanhoenacker, G.; Sandra, P.; Davis, A. P.; De Clercq, P. J. *J. Comb. Chem.* **2002**, *4*, 552–562; (d) De Muynck, H.; Madder, A.; Farcy, N.; De Clercq, P. J.; Pérez-Payán, M. N.; Öhberg, L. M.; Davis, A. P. *Angew. Chem., Int. Ed.* **2000**, *39*, 145–148; (e) Li, H.; Wang, L.-X. *Org. Biol. Chem.* **2003**, *1*, 3507–3513; (f) Albert, D.; Feigel, M. *Helv. Chim. Acta* **1997**, *80*, 2168–2181; (g) Cheng, Y.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 1813–1814; (h) Boyce, R.; Li, G.; Nestler, H. P.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 7955–7956; (i) Wess, G.; Bock, K.; Kleine, H.; Kurz, M.; Guba, W.; Hemmeric, H.; Lopez-Calle, E.; Baringhaus, K.-H.; Glombic, H.; Enhsen, A.; Kramer, W. *Angew. Chem., Int. Ed.* **1996**, *35*, 2222–2224.
7. (a) Davis, A. P. *Molecules* **2007**, *12*, 2106–2122; (b) Davis, A. P. *Coord. Chem. Rev.* **2006**, *250*, 2939–2951; (c) Clare, J. P.; Ayling, A. J.; Joos, J.-B.; Sisson, A. L.; Magro, G.; Pérez-Payán, M. N.; Lambert, T. N.; Shukla, R.; Smith, B. D.; Davis, A. P. *J. Am. Chem. Soc.* **2005**, *127*, 10739–10746; (d) Virtanen, E.; Kolehmainen, E. *Eur. J. Org. Chem.* **2004**, 3385–3399; (e) Davis, A. P.; Joss, J.-B. *Coord. Chem. Rev.* **2003**, *240*, 143–156; (f) Sisson, A. L.; Clare, J. P.; Taylor, L. H.; Charmant, J. P. H.; Davis, A. P. *Chem. Commun.* **2003**, 2246–2247.
8. (a) Albert, D.; Feigel, M.; Benet-Buchholz, J.; Boese, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 2727–2729; (b) Albert, D.; Feigel, M. *Tetrahedron Lett.* **1994**, *35*, 565–568.
9. (a) Feigel, M.; Ladberg, R.; Engels, S.; Herbst-Irmer, R.; Fröhlich, R. *Angew. Chem., Int. Ed.* **2006**, *45*, 5698–5702; (b) Rivera, D. G.; Wessjohann, L. A. *J. Am. Chem. Soc.* **2006**, *128*, 7122–7123; (c) Wessjohann, L. A.; Voigt, B.; Rivera, D. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 4785–4790.
10. (a) Bhattarai, K. M.; del Amo, V.; Magro, G.; Sisson, A. L.; Joss, J.-B.; Charmant, J. P. H.; Kantacha, A.; Davis, A. P. *Chem. Commun.* **2006**, 2335–2337; (b) Kallner, A. *Acta Chem. Scand.* **1967**, *21*, 322–328.
11. (a) Rosenkranz, G.; Mancera, O.; Sondheimer, F.; Djerassi, C. *J. Org. Chem.* **1956**, *21*, 520–522; (b) Ringold, H. J.; Rosenkranz, G.; Sondheimer, F. *J. Am. Chem. Soc.* **1956**, *78*, 820–825; (c) Rosenkranz, G.; Pataki, J.; Djerassi, C. *J. Am. Chem. Soc.* **1951**, *73*, 4055–4056.
12. (a) Rivera, D. G.; León, F.; Coll, F.; Davison, G. P. *Steroids* **2006**, *71*, 1–11; (b) Iglesias-Arteaga, M. A.; Pérez, C. S.; Coll, F. *Steroids* **2002**, *67*, 159–164; (c) Seok, J.; Fuchs, P. L. *Org. Lett.* **2003**, *5*, 2247–2250; (d) Li, W.; Fuchs, P. L. *Org. Lett.* **2003**, *5*, 2849–2852; (e) Gryszkiewicz-Wojtkielewicz, A.; Jastrzębska, I.; Morzycki, J. W.; Romanowska, D. B. *Curr. Org. Chem.* **2003**, *7*, 1–22; (f) Betancor, C.; Freire, R.; Pérez-Martín, I.; Prangé, T.; Zuáres, E. *Org. Lett.* **2002**, *4*, 1295–1297.
13. (a) Iglesias-Arteaga, M. A.; Pérez Martínez, C. S.; Coll Manchado, F. *J. Chem. Res., Synop.* **2001**, 34–35; (b) Zhang, Y.; Li, Y.; Zhu, S.; Guan, H.; Lin, F.; Yu, B. *Carbohydr. Res.* **2004**, *339*, 1753–1759; (c) Suhr, R.; Lahmann, M.; Osacarson, S.; Thiem, F. *Eur. J. Org. Chem.* **2003**, 4003–4011; (d) Fragoso-Luna, L. M.; Frontana-Uribe, B. A.; Cárdenas, J. *Tetrahdron Lett.* **2002**, *43*, 1151–1155; (e) Pettit, G. R.; Bowyer, W. J. *J. Org. Chem.* **1960**, *25*, 84–86.
14. (a) Wessjohann, L. A.; Rivera, D. G.; Coll, F. *J. Org. Chem.* **2006**, *71*, 7521–7526; (b) Rivera, D. G.; Morejón, O.; Coll, F. *Tetrahedron* **2006**, *62*, 8327–8334.
15. (a) Li, M.; Yu, B. *Org. Lett.* **2006**, *8*, 2679–2682; (b) Hu, K.; Yao, X. *Cancer Invest.* **2003**, *21*, 389–393; (c) Hu, K.; Dong, A.; Yao, X.; Kobayashi, H.; Iwasaki, S. *Planta Med.* **1997**, *63*, 161–165; (d) Shao, Y.; Poobrasert, O.; Kenelly, E. J. *Planta Med.* **1997**, *63*, 258–262; (e) Nakamura, T.; Komori, C.; Lee, Y.-y.; Hashimoto, F.; Yahara, S.; Nohara, T.; Ejima, A. *Biol. Pharm. Bull.* **1996**, *19*, 564–566.
16. (a) Iglesias-Arteaga, M. A.; Pérez Gil, R.; Leliebre Lara, V.; Pérez Martínez, C. S.; Coll Manchado, F. *J. Chem. Res., Synop.* **1996**, 504–505; (b) Iglesias-Arteaga, M. A.; Pérez Gil, R.; Leliebre Lara, V.; Pérez Martínez, C. S.; Rosado, A.; Coll Manchado, F. *Synth. Commun.* **1998**, *28*, 1381–1386; (c) Iglesias-Arteaga, M. A.; Pérez Gil, R.; Leliebre Lara, V.; Pérez Martínez, C. S.; Coll Manchado, F. *Synth. Commun.* **1998**, *28*, 1779–1784.
17. Arsenou, E. S.; Koutsourea, A. I.; Fousteris, M. A.; Nikolaropoulos, S. S. *Steroids* **2004**, *68*, 407–414.
18. Zhou, X.-T.; Rehman, A.; Li, C.; Savage, P. B. *Org. Lett.* **2000**, *2*, 3015–3018.
19. For selected reviews, see: (a) Wessjohann, L. A.; Andrade, C. K. Z.; Vercillo, O. E.; Rivera, D. G. *Targets Heterocycl. Systems* **2006**, *10*, 24–63; (b) von Nussbaum, F.; Brands, M.; Hinzen, B.; Weingand, S.; Häbich, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 5072–5129; (c) Gibson, S. E.; Lecci, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 1364–1377; (d) Tan, N.-H.; Zhou, J. *Chem. Rev.* **2006**, *106*, 840–895; (e) Wessjohann, L. A.; Ruijter, E.; Rivera, D. G.; Brandt, W. *Mol. Divers.* **2005**, *9*, 171–186; (f) Hamada, Y.; Shioiri, T. *Chem. Rev.* **2005**, *105*, 4441–4482; (g) Nicolaou, K. C.; Boddy, C. N. C.; Bräse, S.; Winssinger, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 2096–2152.
20. (a) Ueda, S.; Oishi, S.; Wang, Z.; Araki, T.; Tamamura, H.; Cluzeau, J.; Ohno, H.; Kusano, S.; Nakashima, H.; Tren, J. O.; Peiper, S. C.; Fujii, N. *J. Med. Chem.* **2007**, *50*, 192–198; (b) Fletcher, S.; Hamilton, A. D. *J. R. Soc. Interface* **2006**, *3*, 215–233; (c) Ying, J.; Gu, X.; Cai, M.; Dedek, M.; Vagner, J.; Trivedi, D. V.; Hruby, V. J. *J. Med. Chem.* **2006**, *49*, 6888–6896 and references therein; (d) Ye, Y.; Li, W. P.; Anderson, C. J.; Kao, J.; Nikiforovich, G. V.; Achilefu, S. *J. Am. Chem. Soc.* **2003**, *125*, 7766–7767.
21. (a) Marinelli, L.; Lavecchia, A.; Gottschalk, K.-E.; Novellino, E.; Kessler, H. *J. Med. Chem.* **2003**, *46*, 4393–4404; (b) Gottschalk, K.-E.; Kessler, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3767–3774; (c) Goodman, S. L.; Hölzemann, G.; Sulyok, G. A. G.; Kessler, H. *J. Med. Chem.* **2002**, *45*, 1045–1051; (d) Belvisi, L.; Bernardi, A.; Checchia, A.; Manzoni, L.; Potenza, D.; Scolastico, C.; Castorina, M.; Cupelli, A.; Giannini, G.; Carminati, P.; Pisano, C. *Org. Lett.* **2001**, *3*, 1001–1004; (e) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461–7477.
22. (a) Rezai, T.; Yu, B.; Millhauser, G. L.; Jacobson, M. P.; Lokey, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 2510–2511; (b) March, D. R.; Abbenante, G.; Bergman, D. A.; Brinkworth, R. I.; Wickramasinghe, W.; Begun, J.; Martin, J. L.; Fairlie, D. P. *J. Am. Chem. Soc.* **1996**, *118*, 3375–3379; (c) Burton, P. S.; Conradi, R. A.; Ho, N. F. H.; Hilgers, A. R.; Borchardt, R. T. *J. Pharm. Sci.* **1996**, *85*, 1336–1340; (d) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512–523.
23. Hruby, V. J.; al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249–262.
24. Doores, K. J.; Mimura, Y.; Dwek, R. A.; Rudd, P. M.; Elliott, T.; Davis, B. G. *Chem. Commun.* **2006**, 1401–1403.
25. For selected examples, see: (a) Cristau, P.; Martin, M.-T.; Dau, M. T. H.; Vors, J.-P.; Zhu, J. *Org. Lett.* **2004**, *6*, 3183–3186; (b) Finks, B. E.; Kim, P. R.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 4334–4344.