Total Synthesis

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Total Synthesis, NMR Solution Structure, and Binding Model of the Potent Histone Deacetylase Inhibitor FR235222**

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The organization of DNA into chromatin plays a major role in gene regulation. Changes in chromatin architecture (chromatin remodeling) can be modulated by acetylation, methylation, phosphorylation, and ubiquitination of N-terminal histone tails.[1] Histone acetylation is the best-understood mechanism of chromatin remodeling, and is essentially governed by the antagonistic activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs).[2] As acetylation is a key component of gene expression, HAT and HDAC inhibitors or activators have been studied as potential therapeutic agents.[3] In the frame of the various histone acetylase inhibitors, natural cyclopeptides play an important role as reversible or irreversible zinc chelators with effective anticancer and antiprotozoal activities. The conformational features of selected members of this class of compounds (for example, apicidin and congeners) have been established by X-ray and NMR spectroscopic studies and by comparison of their biological activity with that of simplified synthetic analogues.[4]

Cyclopeptide FR235222 (**1**; see Scheme 2), which was recently isolated from the fermentation broth of *Acremonium* sp.,[5] showed a potent and selective inhibition of T cell

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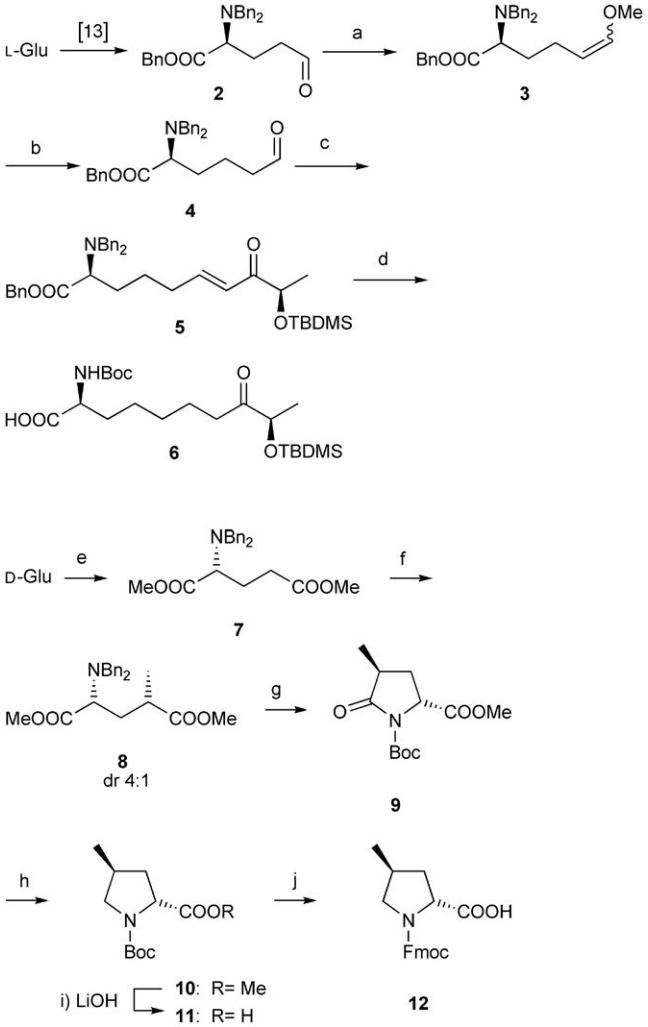
proliferation and lymphokine production, and also exhibited a potent inhibition of HDAC.[6] This immunosuppressant activity highlights **1** as an attractive target for chemical synthesis and, in fact, Ma and co-workers recently reported its total synthesis.[7] The availability of a synthetic sample of **1** was also deemed important to examine its HDAC binding features, taking into account that details of the determinants for the recognition between cyclopeptides and HDAC enzymes are only partly and indirectly known. Finally, besides the inherent interest evoked by its biological properties, we envisaged that a convergent synthetic approach, combined with key information on its bioactive conformation and HDAC binding interaction, would pave the way to the assembly of focused libraries of simplified agents and to the rational design of more powerful and selective analogues.

Hence, in the context of our continuous efforts in synthesizing peptide-related molecules,[8] and animated by the opportunities that would result in terms of chemical biology and medicinal chemistry studies,[9] we looked for an effective and possibly flexible route to **1**. Even at first glance it is apparent that the most difficult task, from a synthetic viewpoint, resides in the stereoselective access to the two key noncoded amino acids present in **1**, namely (2*S*,9*R*)-2-amino-9-hydroxy-8-oxodecanoic acid (Ahoda) and *trans*-4-methyl-D-proline, in the form of suitably protected building blocks for their subsequent incorporation into standard solid-phase peptide chemistry protocols.

Ahoda, the long-chain α-amino acid residue, is a typical structural element of class I and II HDAC peptide inhibitors,[3b] all of which invariably present one of such substrate-analogue moieties,[10] with the function to chelate the Zn^{II} ion in the active site. Although Ahoda is present in many other cyclopeptide HDAC inhibitors,[4b,11] only one synthesis has been reported to date.[7,12] We decided to follow a retrosynthetic approach, in which the configuration at C2 was derived from L-glutamic acid and the configuration at C9 was derived from (*R*)-lactic acid. Thus, compound **2**, prepared as previously reported,[13] was homologated to the six-carbon-chain aldehyde **4** via the enol ether **3** (Scheme 1). Wittig–Horner–Emmons reaction with dimethyl (*R*)-[3-(*tert*-butyldimethylsilyloxy)-2-oxobutyl]phosphonate[14] gave compound **5** in good yield as a single diastereoisomer. Reduction and contemporaneous protecting-group exchange was carried out by catalytic hydrogenation (in a Parr apparatus) to give *N*-Boc-*O*-TBDMS-Ahoda (**6**), ready for use in peptide synthesis. HPLC and 600-MHz ^{1}H NMR analyses of **6** established that racemization did not occur along the full synthetic pathway.

Substituted prolines have been extensively investigated as rigid elements for conformationally restricted peptidomimetics, and many synthetic procedures have been described to implement a wide variety of functional groups on pyrrolidine-2-carboxy derivatives.[15] In particular, 4-methylproline (4-MePro), which is also present in other natural products,[16] has been recently prepared by stereocontrolled hydrogenation of the corresponding methylene derivative obtained from 4-hydroxyproline.[17] Appealing as it is, with its use of a naturally occurring starting material, this method becomes economically demanding when access to 4-MePro of the D series is desired, as in our case. In an attempt to rationalize our needs



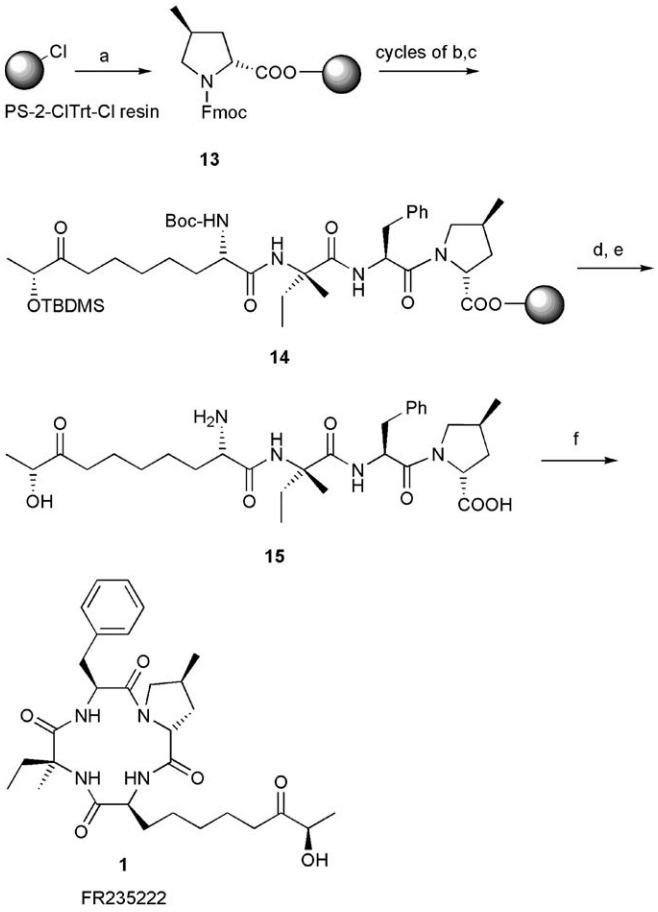
Scheme 1. Reagents and conditions: a) $MeOCH_2PPh_3Cl$, KHDMS, THF, 0 °C; 75%; b) HCl/AcOEt; 94%; c) (*R*)-$(MeO)_2P(O)CH_2COCH(O$-TBDMS)CH_3, LiCl, DIEA, MeCN; 87%; d) $Pd(OH)_2$, H_2 (6 atm), Boc_2O, MeOH; 75%; e) BnBr, NaOH, Na_2CO_3, H_2O followed by $SOCl_2$ in MeOH; 76%; f) KHMDS, THF, MeI, −78 °C; 92%; g) $Pd(OH)_2$, H_2 (6 atm), MeOH, then Boc_2O, Et_3N, DMAP, MeCN followed by column chromatography; 60%; h) $BH_3{\cdot}Me_2S$ in THF, 40 °C, 72 h; 60%; i) LiOH, THF, H_2O; j) TFA/CH_2Cl_2, TIS, followed by FmocOSu, Na_2CO_3, H_2O; 77%. KHMDS = potassium 1,1,1,3,3,3-hexamethyldisilazane, TBDMS = *tert*-butyldimethylsilyl, DIEA = ethyldiisopropylamine, Boc = *tert*-butyloxycarbonyl, Bn = benzyl, DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, TIS = triisopropylsilane, FmocOSu = 9-fluorenylmethyloxycarbonyl-*N*-hydroxysuccinimide.

in terms of the chiral pool,[18] we sought an efficient route by starting again from glutamic acid. Clearly, a key step was the stereoselective preparation of (2*R*,4*S*)-4-MeGlu.

Special care was taken in the selection of the protection scheme, as it was known that the NHBoc group strongly favors the attack of MeI on the opposite side of the Glu γ-enolate, as a consequence of a chelat-ion-controlled transition state.[19] Assuming that the formation of another anionic site at the nitrogen atom was therefore detrimental for our purposes, we found that the *N*,*N*-dibenzyl dimethyl ester **7** could be stereoselectively methylated in position 4 to give **8** as a 4:1 mixture of diastereomers.[20] Upon catalytic hydro-

genation, **8** directly produced the ring-closure lactam, from which, after *N*-Boc protection, the major isomer **9** was isolated by column chromatography (60% yield). The configuration of **9** was determined by comparison with previously reported NMR data.[15,21] Chemoselective reduction of the lactam carbonyl group, followed by ester hydrolysis and Boc/Fmoc protecting-group exchange, finally gave the required Fmoc-amino acid **12** in three steps (46% yield).

With compounds **6** and **12** in hand, the synthesis of **1** was attempted by following a typical solid-phase peptide chemistry protocol (Scheme 2), using a 2-chlorotrityl resin.[22] Fmoc-D-4-MePro was loaded on the resin and followed by HATU/HOBT promoted couplings with L-Phe and L-Iva. Product **6** was introduced as the last amino acid before cleavage from the resin with AcOH/TFE/TIS, which gave the linear precursor (**15**) of FR235222 in 70% yield. After removal of TBDMS and Boc groups from Ahoda by treatment with TFA/H_2O/TIS (95% yield),[23] the unprotected linear tetrapeptide was cyclized by exposure to HATU (2 equiv) and DIEA (2 equiv) in a very dilute solution to avoid side formation of the cyclodimeric sequence of **1** as by-product. The cyclized product was obtained in 68% yield after purification by HPLC. The analytical data ($[\alpha]_D$, electrospray mass spectrometry, ^1H and ^{13}C NMR spectroscopy) measured on the synthetic sample were virtually indistinguishable from those reported for the natural product.[24]



Scheme 2. Final stages of the total synthesis of **1**: a) DIEA, CH_2Cl_2; b) 20% piperidine, DMF; c) Fmoc-AA or Ahoda derivative **6**, HOBT, HBTU, NMM, DMF; d) AcOH/TFE/CH_2Cl_2 (2:2:6); e) TFA/H_2O/TIS (94:4:1); f) HATU, CH_2Cl_2, $c = 7.7 \times 10^{-5}$ M. DIEA = *N*,*N*-diisopropylethylamine, DMF = *N*,*N*-dimethylformamide, AA = amino acid, HOBT = *N*-hydroxybenzotriazole, HBTU = *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, NMM = *N*-methylmorpholine, TFE = 2,2,2-trifluoroethanol, PS-2-ClTrt-Cl resin = polystyrene/2-chlorotrityl chloride resin.

Next we determined the solution conformation of **1**, a logical prerequisite for the subsequent analysis of the nature of its binding interactions with HDAC enzymes. Although cyclopeptides represent an important class of HDAC inhibitors with potential activity as antitumor and antiprotozoal compounds, no relevant information on the type and nature of the molecular determinants for their interactions with HDAC isozymes has yet been reported. The crystal structures of HDAC–ligand complexes are, in fact, limited to small-molecule inhibitors, such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), with no examples of inhibitors with a large head (cap) group.[25] A conformational analysis of **1** was carried out by restrained molecular mechanics (MM) and dynamics (MD) calculations (1 ns, see Supporting Information).

The solution structure depicted in Figure 1 was obtained by using 21 distance constraints, previously collected in a series of ROESY spectra ($CDCl_3$ and [D_6]DMSO, 600 MHz, $t_{mix} = 50$, 100, and 150 ms). Visual inspection of this conformation shows that the molecule consists of a central core, which comprises the tetrapeptide backbone, the methylproline (MePro) ring, and the whole isovaline moiety, from which phenylalanine and Ahoda side-chain appendages project facing in opposite directions (Figure 1). The MePro residue is characterized by a *trans* geometry of its peptide linkage, as witnessed by an intense ROESY cross-peak between H^{α} of Phe and $H^{\delta}{}_2$ of MePro. The tetrapeptide backbone describes a γ turn and a γ inverse turn, as indicated by the ϕ/ψ torsion angle values (4-MePro: +76/−83; Ahoda: +78/−60; Iva: −89/+93; Phe: −108/+102). Three hydrogen-bonding interactions, all between residues i and $i+2$, stabilize the two γ turns. Thus, the Phe-CO and NH moieties are H-bonded to Ahoda-NH and CO (2.09 and 1.92 Å, respectively), whereas 4-MePro-CO is weakly H-bonded to Iva-NH (2.7 Å).

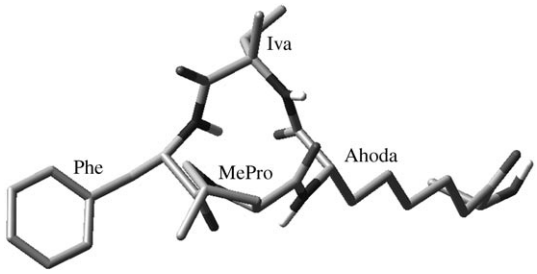


Figure 1. NMR solution conformation of FR235222 (**1**) obtained by restrained MD calculations by using ROESY ($t_{mix} = 100$ ms) data collected at 600 MHz in $CDCl_3$ and [D_6]DMSO.

The next step was a docking study between FR235222 (**1**) and HDAC with the software ICM 3.2 (MolSoft LLC,

La Jolla, CA), which takes advantage of a particularly efficient proprietary docking algorithm. For ICM docking the receptor is represented by a series of grid potentials, and both ligand and receptor are flexible and explicit molecules. Moves of ligand and receptor (side chain and loops) are computed on the basis of pseudo-Brownian dynamics.[26] The docking procedure was first validated by reproducing TSA positioning in the histone deacetylase-like protein (HDLP) binding pocket, with the TSA–HDLP complex (3D coordinates: PDB archive code 1C3R) as reference.[25,27] As the final root-mean-square deviation (RMSD) computed on TSA heavy atoms (docked versus X-ray) was less than 0.1 Å, we proceeded further on docking FR235222. Figure 2 shows an

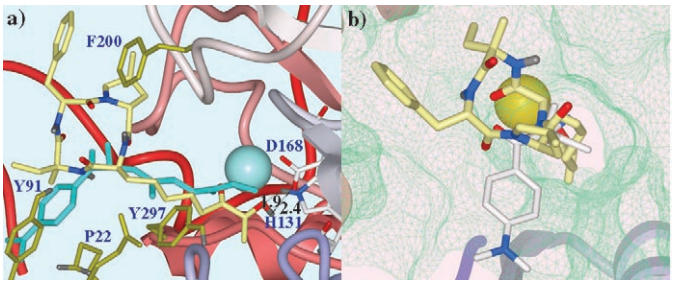


Figure 2. a) 3D model of the interaction between FR235222 (**1**) and HDLP. For a more convenient visual inspection of the positioning of **1** in the active site, TSA is also displayed in cyan. The protein is represented by ribbons (blue to red, N to C terminus) with only a selection of side chains shown. Gold: side chains externally delimiting the binding site; white: those involved in H-bonding interactions (black dashed lines with distances in Å). Only heavy atoms are displayed, except for polar hydrogen atoms (gray). The Zn^{II} ion is represented by a CPK sphere in light blue. b) The different kinds of surface complementarity exhibited by TSA (carbon atoms: white) and FR235222 (carbon atoms: yellow) cap groups toward HDLP are shown. The HDLP molecular surface is depicted as green wire frames (see also text).

expanded view of the model of FR235222 bound to the active site of HDLP. To allow a meaningful comparison, TSA is also displayed in the same original positioning as that found in the crystallographic structure of the TSA–HDLP complex. Notably, this 3D model highlights the fact that, although substantially different in cap groups, both TSA and FR235222 project their zinc-chelating elements in a very similar fashion.

Among the principal polar interactions that stabilize the FR235222 molecule within the HDAC binding pocket, the two oxygen atoms of the α-hydroxyketone terminal function of Ahoda embrace the metal ion, with the hydroxy group also involved in two key ligand–protein hydrogen bonds with H131 and D168, two of the main residues taking an active role in the enzymatic catalysis (see Figure 2a). Van der Waals interactions are also of great importance in the FR235222–HDLP recognition process, especially for the accommodation of the cap group. Indeed, the tetrapeptide cap group of **1** and the outward side of the enzyme exhibit complementary surfaces that correspond with the rim of the tubular pocket that leads to the zinc ion, the latter being filled by the Ahoda long chain.

Also remarkable is the difference of positioning between the TSA and FR235222 head groups (Figure 2b). On the basis of such a model, our hypothesis is that cyclic tetrapeptide inhibitors of this kind are able to bind with high affinity to HDAC because there is a large, shallow cavity that can accommodate the globe-shaped tetrapeptide core, thus allowing the substrate-mimetic chain to extend and reach the zinc ion. In more detail, the phenyl group of TSA is bent toward Y91 (E98 in HDAC1) and P22 (P29), whereas the FR235222 macrolactam domain is projected on the other side, and fills a cavity delimited by F200 and Y91 (Figure 2a). The role of the methyl group of 4-MePro raises the speculation that more bulky groups at this position could potentially allow additional van der Waals and hydrophobic interactions with the protein surface. A final consideration relates to the backbone features of the cyclopeptide core. The consecutive γ turn and γ inverse turn are certainly essential for the correct global shape of the core and for the positioning of the Ahoda side chain. This should be taken into account for the design of simplified active analogues.

On the basis of this model it appears that, besides the macrolactam scaffold, three quite voluminous appendages may be housed within the cavity between F200 and Y91. In other words, there should be quite a wide range of allowed decorations that may conveniently replace the Phe and Iva residues of FR235222, a finding partly confirmed by structure–activity relationship data on cyclopeptide HDAC inhibitors of this type.[28] In a rational design of new peptidomimetic HDAC inhibitors, assuming that the zinc-binding element can be easily implemented by a variety of different solutions,[10] novel scaffolds should be selected from among rigid mono- or bicyclic elements bearing two or three decorating appendages. These should preferably be aromatic side chains to take advantage of the location of F200 and Y91, two sites for favorable π–π stacking interactions.

In conclusion, we have synthesized FR235222 (**1**) by using a convergent approach, classical solid-phase peptide synthesis (SPPS) protocols and macrolactamization in dilute solution. Two non-proteinogenic residues, Ahoda and 4-MePro, were stereoselectively prepared in suitably protected forms for their subsequent employment in SPPS. In addition, we have studied the NMR solution structure of **1** and, more importantly, carried out docking studies with HDAC-like protein. Our integrated study allowed us to derive a 3D model for cyclopeptide interaction with the active site of HDAC, thus highlighting critical differences between the binding modes of small-molecule and cyclopeptide inhibitors.

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