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ANALOGUES OF TRICHOSTATIN A AND TRAPOXIN B AS HISTONE DEACETYLASE INHIBITORS#

Manfred Jung[a]*, Katharina Hoffmann[a], Gerald Brosch[b] and Peter Loidl[b]

[a]Department of Pharmaceutical Chemistry, University of Münster, Hittorfstr. 58-62, 48149 Münster, Germany
[b]Dept. of Microbiology, Medical School, University of Innsbruck, Fritz-Pregl-Str. 3, 6020 Innsbruck, Austria

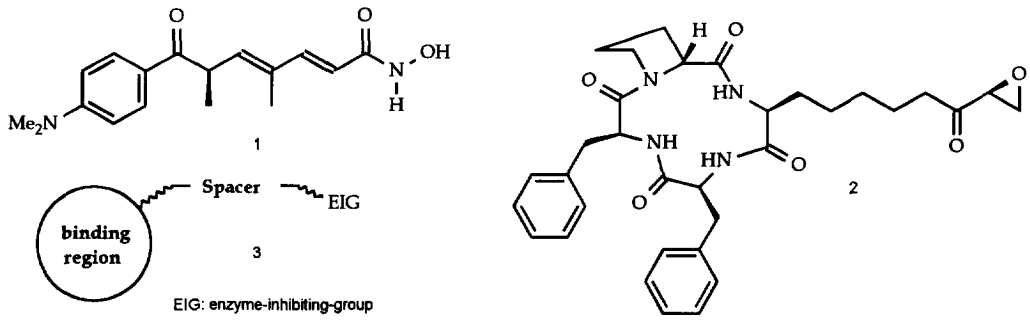
Abstract: Inhibitors of histone deacetylase are potent inducers of differentiation and bear considerable potential as drugs for chemoprevention and treatment of cancer. So far only complex natural products and a few synthetic congeners have been identified as specific inhibitors. We have prepared a set of simple analogues in as little as four synthetic steps that have inhibitory potencies in the range of known cyclotetrapeptide inhibitors. These compounds are interesting leads for the design of potent inhibitors of histone deacetylase. © 1997 Elsevier Science Ltd.

Histones are the protein component of eukaryotic chromatin. The reversible acetylation of distinct lysine residues in the N-terminal tails of core histones contributes to the regulation of nuclear processes, as DNA replication[1], transcription[2] and differentiation[3]. The modification is established and maintained by histone acetyltransferases and histone deacetylases. Inhibition of histone deacetylase leads to hyperacetylation of core histones, thereby affecting growth and differentiation of cells[4]. It has long been recognized that inhibition of histone deacetylase by specific and unspecific inhibitors leads to terminal differentiation of leukaemic cells[5], a process that could be explored for the prevention and treatment of cancer[6]. Other inducers of differentiation such as retinoids and vitamin-D-derivatives are already established in cancer therapy and are investigated in clinical trials for cancer chemoprevention[7].

Only few inhibitors of histone deacetylase have been reported. Sodium butyrate is a non-specific inhibitor in the millimolar range[8] and some butyrate analogues have been reported to have similar activity[9]. There are several natural products which have been identified as potent and specific inhibitors of histone deacetylase. One of them is trichostatin A **1** which possesses potent antifungal activity besides its differentiating properties[5]. The other belong all to a set of hydrophobic cyclotetrapeptides such as trapoxin B **2**[10a].

Dedicated to Prof. Dr. G. Blaschke on the occasion of his 60th birthday

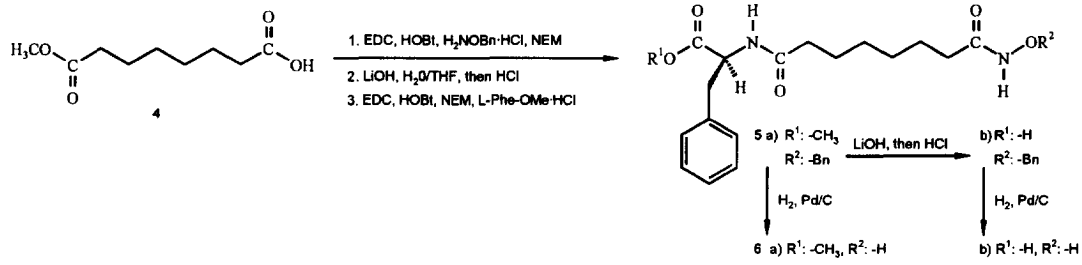
[a] Fax: 49-251-83-335, E-Mail: jungm@uni-muenster.de
[b] Fax: 43-512-507-2866, E-Mail: Peter.Loidl@uibk.ac.at



Scheme 1. Trichostatin A **1**, Trapoxin B **2**, general structure for inhibitors **3**

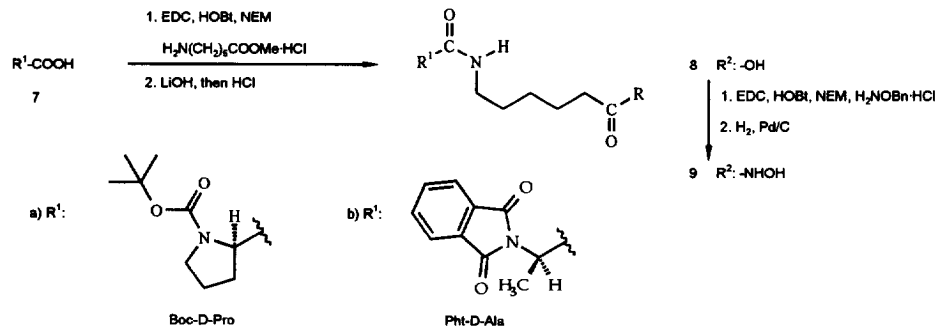
We wanted to combine structural elements of **1** and **2** to get to simple and strong inhibitors of histone deacetylase as so far only a few tetrapeptide analogues used in the isolation and microsequencing of the bovine enzyme are known as potent synthetic inhibitors[10]. The (S)-antipode of the natural trichostatin A and the respective carboxylic acids have been prepared and all were found to be inactive[11]. We propose a general structure for potential inhibitors **3** where a binding region which is responsible for enzyme specifity is separated by a spacer from a group which effects inactivation of the enzyme. We set out to combine various elements from **1** and **2** according to this general structure as in scheme 1.

The synthesis of the carboxylic acid amide-hydroxamates **6** started from mono methyl octanedioate **4** and standard peptide coupling techniques led to the desired products in four (**6a**) or five (**6b**) steps (scheme 2). **6b** could only be isolated in a pure and crystalline form after purification of a small sample on preparative RP-TLC[12].



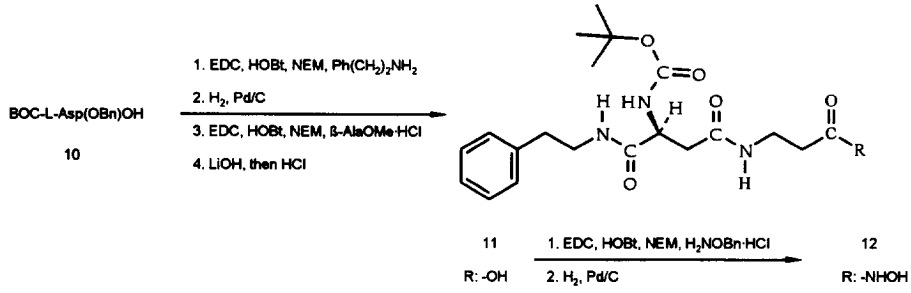
Scheme 2. Synthesis of **6**.

The ω-amino acid hydroxamates **9** were prepared similarily from the protected amino acids **7** (scheme 3).



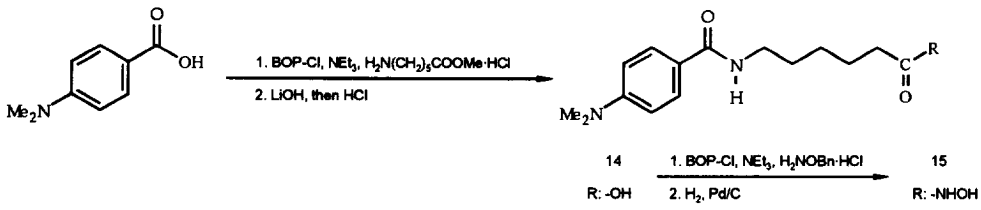
Scheme 3. Synthesis of **9**

The synthesis of analogue **12** in which an α-amino acid was supposed to mimic the enzyme binding region was accomplished from the suitably protected aspartate **10** (scheme 4). The spacer contains an additional peptide bond which could probe for hydrogen bonding near the active site of the enzyme.



Scheme 4. Synthesis of **12**

Finally we replaced the α-methyl ketone unit in trichostatin A **1** by an amide bond which resulted in analogue **15**. Attempts to use carbodiimide or mixed anhydride coupling techniques with *para*-dimethylamino benzoic acid **13** failed, probably due to the highly deactivating influence of the *para*-substituent. But we succeeded using BOP-Cl[13] as the coupling reagent (scheme 5).



Scheme 5. Synthesis of **15**

The inhibitory potency of the hydroxamates **6**, **9**, **12** and **15** and the carboxylic acids **8**, **11** and **14** was tested using maize histone deacetylases as published previously[14]. All carboxylic acids and the proline and

alanine derived hydroxamates **9** were inactive at 30 μM. In contrast the phenylalanine derivatives **6** and the dimethylaminobenzoic acid amide **15** were potent inhibitors of pure deacetylase HD 2[15] (at 30 μM: **6a** 76 % inhibition; **6b** 82 %; **15** 94 %[16]). These inhibitory concentrations are in the range of various cyclotetrapeptides (IC_{90} = 10 μM[14])[17], but still two orders of magnitude above **1** (IC_{90} = 0.1 μM[14]). Thus synthetic non-cyclotetrapeptide inhibitors of histone deacetylase **5** and **12** can be obtained by an facile synthetic sequence in four steps. These are promising findings on the way to simple nanomolar inhibitors of histone deacetylase.

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