Amide Analogues of Trichostatin A as Inhibitors of Histone Deacetylase and Inducers of Terminal Cell Differentiation

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Received May 31, 1999

Inhibitors of histone deacetylase (HD) bear great potential as new drugs due to their ability to modulate transcription and to induce apoptosis or differentiation in cancer cells. We have described previously analogues of the complex natural HD inhibitors trapoxin B and trichostatin A with activities in the submicromolar range. Here we report structure−activity relationship analyses of further analogues of trichostatin A with respect to in vitro inhibition of maize HD-2 and their ability to induce terminal cell differentiation in Friend leukemic cells. This is the first report that shows the correlation between HD inhibitory activity and action on cancer cells on a larger series of similar compounds. Only the compounds that inhibit HD induce differentiation and/or exert antiproliferative activities in cell culture. Our studies support the use of in vitro systems as screening tools and provide structure−activity relationships that merit further investigation of this interesting target.

Introduction

Key nuclear processes such as DNA replication, transcription, repair, and rearrangements during differentiation are influenced by chromatin structure and the binding of regulatory proteins to DNA. These processes can be modulated by the acetylation level of the ϵ-amino groups of highly conserved lysine residues in the N-terminal tails of nucleosomal histones.[1] This level is established and maintained by histone acetyltransferases and histone deacetylases (HDs[2]), enzymes which have been recently identified as conserved homologues of transcriptional regulators and nucleolar phosphoproteins.[3−5] Increasing evidence indicates that cellular proteins involved in the regulation of proliferation and differentiation exert their function by recruitment of histone acetyltransferases or deacetylases.[3,4] In various cases aberrant histone acetylation has been linked to malignant disease.[6−9]

In the course of our studies to synthesize simple inhibitors of HD and to exploit their potential for cancer therapy and chemoprevention we have postulated a general structure for inhibitors of HD **3** (Chart 1), and consequently identified promising lead substances **4** and **5a** according to that postulate.[10] These are derived from structural elements of the potent inhibitors trapoxin B **(1)** and trichostatin A **(2)** that are available only from natural sources in small amounts or by multistep syntheses. In this study we wanted to investigate structure−activity relationships (SARs) for analogues with the general structure **5** and their ability to inhibit HD. We further determined the correlation between enzyme inhibition in vitro and induction of terminal cell differentiation which has so far only been shown for single or few representatives of different types of inhibitors. We present a series of novel inhibitors of HD with potential to induce cell differentiation. These compounds can be considered promising agents in investigating the role of histone acetylation in regulation of cell growth and in cancer therapy and chemoprevention.

Chemistry

For the synthesis of amide analogues of **2**, we chose a four-step strategy that allows for variation in the specificity region, the lipophilic spacer, and possibly the functional group responsible for enzyme inhibition according to our postulated general structure for inhibitors **3**. In the first step an aromatic acid representing the dimethylaminobenzoyl group in **2** is coupled with an Ω-amino acid spacer derived from **1** by using BOP-Cl or acid chloride activation. For 4-dimethylaminobenzoic acid, we had proven previously that carbodiimide or mixed anhydride activation fails, probably due to the high deactivation of the acyl reactivity by the 4-dimethylamino substituent.[10] The resulting esters **6** were cleaved to the acids **7** which were included in the screening as well (Scheme 1).

For the first series of structural modifications we had chosen to use the hydroxamate as the inactivating functional group because it was known for the highly potent trichostatin A **(2)** that its activity is dependent on that function.[11] As the final products are very polar and difficult to chromatograph, we followed a synthetic pathway that involves the use of a properly protected hydroxylamine. The resulting precursor to the target structure can be easily purified by flash chromatography. The final deprotection step was selected in such a fashion that no further chromatography was necessary. Wherever possible we used the benzyl protecting group

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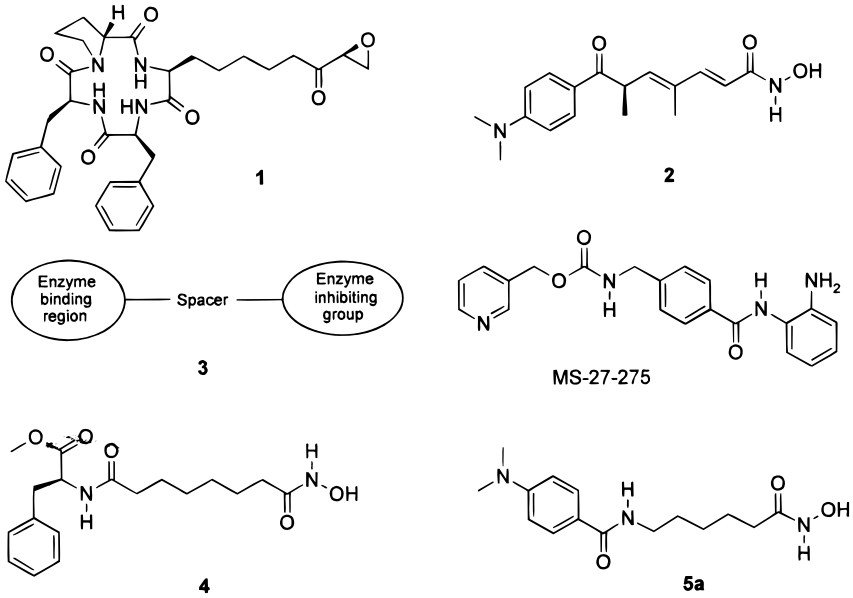
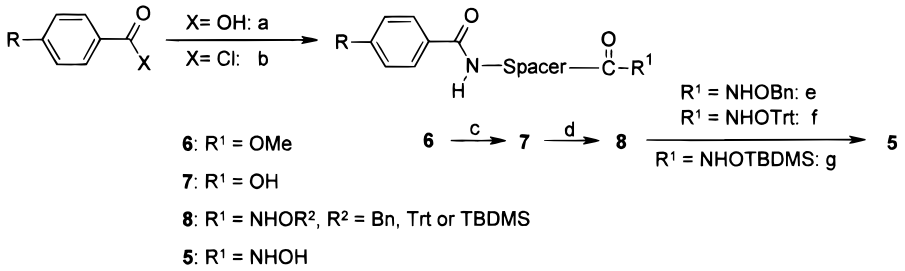
10.1021/jm991091h CCC: $18.00
Published on Web 10/15/1999

Chart 1. Inhibitors of HD



Scheme 1[a]



[a] TEA, BOP-Cl, H_2N-spacer-COOMe·HCl, CH_2Cl_2; (b) TEA, H_2N-spacer-COOMe·HCl, CH_2Cl_2; (c) LiOH, THF, then HCl; (d) TEA, BOP-Cl, H_2N-OBn·HCl or H_2N-OTrt or H_2N-OTBDMS, CH_2Cl_2; (e) H_2, Pd/C, methanol; (f) TFA, Et_3SiH, CH_2Cl_2; (g) TBAF, THF.

and subsequent hydrogenation. When functional groups in the molecule prohibited this, we have also employed TBDMS (**g**) and trityl (**j, k**) protection successfully. Deprotection of an *O*-allyl hydroxamate by palladium catalysis in an attempted synthesis of **5g** provided some of the desired product, but we were unable to isolate it from the crude reaction mixture. In the examples provided here, the trityl group was the better alternative to the benzyl derivatives when necessary. This first study puts its emphasis on demonstrating the validity of the amide bioisostere replacement in our lead structure as compared to trichostatin, the comparison of hydroxamic and carboxylic acid analogues, and the variation of the spacer length. There are also examples on other functional groups rather than the 4-dimethylamino substituent present in **2** and our first lead structure **5a**. This substituent limits desirable synthetic operations that lead to ketone analogues of **2** due to its highly deactivating influence on benzoic acid or benzaldehyde building blocks (e.g. in asymmetric allylation) and its susceptibility to oxidation (e.g. in Sharpless oxidation or dihydroxylation). Generally we provide an easy four-step synthesis that allows access to a variety of inhibitors in larger quantities that are necessary for mechanistic studies. The ester or acid intermediates possibly allow the introduction of functional groups other than hydroxamate which is the subject of ongoing studies. Especially interesting in that regard is the recently discovered class of benzamide inhibitors such as MS-27-275 (Chart 1) that showed oral activity in mouse tumor models.[12,13]

Result

In Vitro Enzyme Inhibition. We used chromatographically purified maize histone deacetylase HD-2 as the enzyme source.[14] In this study various hydroxamates **5** and the corresponding carboxylic acids **7** were tested for their ability to inhibit HD and trichostatin A **(2)** was used as a reference. We first evaluated the variation of the spacer in our lead compound **5a** and then modified the para-substituent in the benzoyl moiety (Table 1).

The inhibitory activity was clearly dependent on the spacer length with a maximum of activity at five and six methylene groups, respectively. The activity drops drastically when the spacer becomes shorter than four methylene groups. Among the constrained analogues **5f** and **5g**, only the benzoic acid congener **5g** demonstrated an inhibitory activity. The acylated aminomethylbenzamide in **5g** is also a structural element of the just recently discovered MS-27-275 inhibitor (Chart 1). The caproic acid spacer from **5a** was chosen for further variation of the para-substituent on the benzamide moiety.

The influence of the substituent was not significant as all compounds demonstrated inhibitory activity in the range of 100 nM to 1 μM. As already described for **2** all

Table 1. Inhibitory Potency of Amide Analogues **5** and **7** of **2**

no.	R	spacer	R^1	$IC_{50} \pm SD$ (nM)
2				3 ± 0.09
5a	Me_2N	$(CH_2)_5$	NHOH	100 ± 20
7a	Me_2N	$(CH_2)_5$	OH	NA[a]
5b	Me_2N	$(CH_2)_3$	NHOH	NA
7b	Me_2N	$(CH_2)_3$	OH	NA
5c	Me_2N	$(CH_2)_4$	NHOH	2000 ± 95
7c	Me_2N	$(CH_2)_4$	OH	NA
5d	Me_2N	$(CH_2)_6$	NHOH	100 ± 4
7d	Me_2N	$(CH_2)_6$	OH	NA
5e	Me_2N	$(CH_2)_7$	NHOH	300 ± 8
7e	Me_2N	$(CH_2)_7$	OH	NA
5f	Me_2N	4-Ph-CH_2	NHOH	NA
7f	Me_2N	4-Ph-CH_2	OH	NA
5g	Me_2N	CH_2-4-Ph	NHOH	180 ± 5
7g	Me_2N	CH_2-4-Ph	OH	NA
5h	MeO	$(CH_2)_5$	NHOH	140 ± 10
7h	MeO	$(CH_2)_5$	OH	NA
5i	H	$(CH_2)_5$	NHOH	900 ± 25
7i	H	$(CH_2)_5$	OH	NA
5j	Cl	$(CH_2)_5$	NHOH	150 ± 5
7j	Cl	$(CH_2)_5$	OH	NA
5k	O_2N	$(CH_2)_5$	NHOH	180 ± 15
7k	O_2N	$(CH_2)_5$	OH	NA
5l	Ph	$(CH_2)_5$	NHOH	100 ± 4
7l	Ph	$(CH_2)_5$	OH	NA

[a] NA, not active at starting concentration of 40 μM.

carboxylic acids were inactive. From the synthetic point of view it is interesting that the methoxy compound **5h**, the chloro analogue **5j**, the nitro compound **5k**, and the biphenyl derivative **5l** were identified as good inhibitors. These may serve as new lead compounds for further structural modifications in the spacer that are difficult to realize in the dimethylamino series.

Induction of Differentiation. In addition to the inhibition of HD activity in an enzyme assay, we investigated the potential of compounds **5** and **7** to induce terminal cell differentiation in the Friend leukemia cell line (mouse erythroleukemic cells, MELC). It has been demonstrated previously that these cells respond to inhibitors of HD such as **2**[15] or the so-called hybrid polar compound SAHA[16] by accumulation of hemoglobin, which is visualized and quantified by benzidine staining. Initial tests were performed at 10 and 50 μM, and dose−response studies followed according to those initial results. If the compounds were highly toxic at 50 μM (>95%) dose−response studies were not extended to that concentration (Figure 1).

Similar to the results obtained regarding the inhibition of HD enzymatic activity, the carboxylic acids **7** were inactive with respect to induction of cell differentiation. The hydroxamic acid derivatives **5** could be divided in five groups according to their influence on cell differentiation and proliferation. In the concentration range tested, compounds **5h** and **5l** were moderate growth inhibitors but did not induce cell differentiation in MEL cells. Compounds **5b** and **5f** had no influence on cell proliferation. With increasing concentrations, they cause weak induction of cell differentiation. Up to 20% of the treated cells were benzidine-positive. The latter are the compounds that did not inhibit maize HD-2 in the assay using labeled histones in concentrations up to 40 μM. In the third group, compounds **5c** and **5k** caused a gradual inhibition of cell growth up to 50 μM. Within the same concentration range, these compounds induced cell differentiation up to 50%. Trichostatin **(2)** displayed a similar pattern of activity; however, the concentration range tested was 1000-fold lower. The fourth group (**5a**, **5e**, and **5i**) had a more pronounced effect on cell proliferation than on cell differentiation. At concentrations above 10 μM the compounds were toxic, but a maximum of only 20% of the surviving cell population were induced to differentiate. Similarly, compounds in group 5 (**5d**, **5g**, and **5j**) were toxic at concentrations above 10 μM. However, these compounds can be regarded as good inducers of cell differentiation, as up to 70% of the cells stained benzidine-positive. It should be noted that even in this group the concentration range in which induction of cell differentiation is measurable is quite narrow. Inhibition of HD enzymatic activity in vitro did not closely correlate with the induction of cell differentiation in MEL cells. As an example, compounds **5h** and **5l** were identified as good inhibitors of HD activity with IC_{50} values of 100 and 140 nM, respectively, but they failed to induce cell differentiation. In the dimethylamino series the relative ranking with regard to inhibition of maize HD-2 was retained in the differentiation assay: e.g. **5d** was among the best inhibitors of the maize enzyme (IC_{50} = 100 nM) and was able to induce a maximum of 70% of the cells to differentiate at 600 nM. Especially interesting is also the chloro compound **5j** as it is the only one that does not contain the dimethylamino substituent but is still able to induce differentiation at lower concentrations. Thus, it is the best starting point for synthetic variations that are difficult in the dimethylamino series.

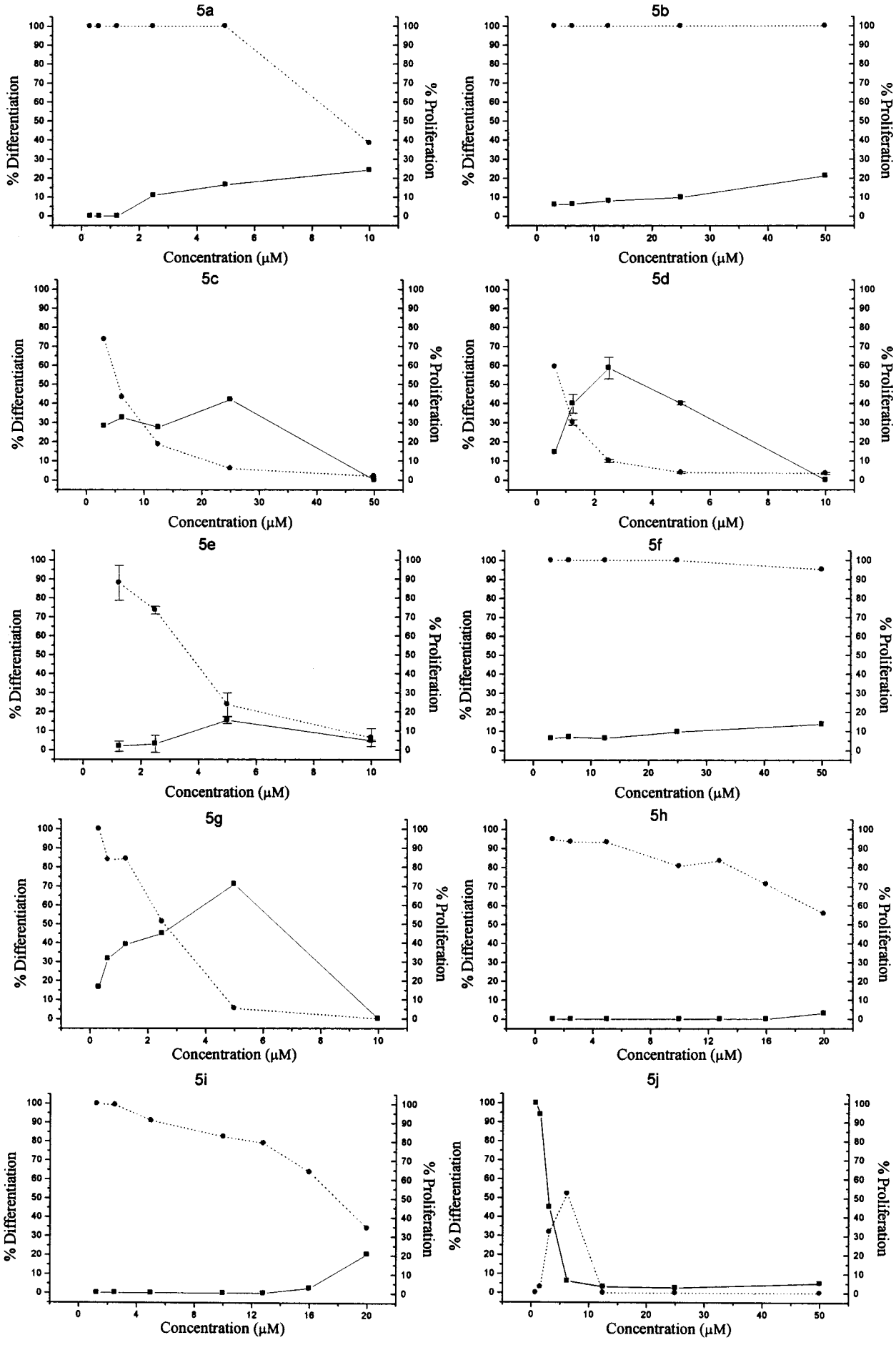
Histone Hyperacetylation. To investigate whether inhibition of HD correlated with induction of cell differentiation in MEL cells, compounds **4**, **5a**, **5d**, **5j**, and **5l** were chosen along with **2** as a reference to study the acetylation level of core histones by gel electrophoresis. The MEL cells were treated with the agents at the indicated concentrations for 6 h. Nuclei and subsequently histones were isolated by standard procedures. Hyperacetylation, especially of histone H4, was then studied after electrophoretic separation employing AUT (acid-urea-Triton-X-100) gels as described in the literature (Figure 2).[17,18]

A clear shift to hyperacetylated species of histone H4 can be seen by incubation with reference compound **2** and the synthetic inhibitors **4**, **5a**, **5d, 5j**, and **5l** indicating an inhibition of HD enzymatic activity in MEL cells. Solvent control shows mainly non- and monoacetylated species, so these compounds are indeed inhibitors of HD in Friend leukemic cells.

Discussion

We have shown that simple potent inhibitors of HD are accessible in a simple four-step synthetic sequence. They are built from structural elements of different types of inhibitors of that enzyme which indicates their common function as acetyl-lysine mimics and validates our postulated general structure **3** for inhibitors of that enzyme.

We have demonstrated a clear dependence of the activity on the spacer length with a maximum of inhibiton at five, respectively, six methylene groups which again points to a competition with an acetyl-lysine. Of the rigid compounds tested, only the substituted benzoyl hydroxamate **5g** was active. This differ-


5a
5b
5c
5d
5e
5f
5g
5h
5i
5j
% Differentiation
% Proliferation
Concentration (μM)

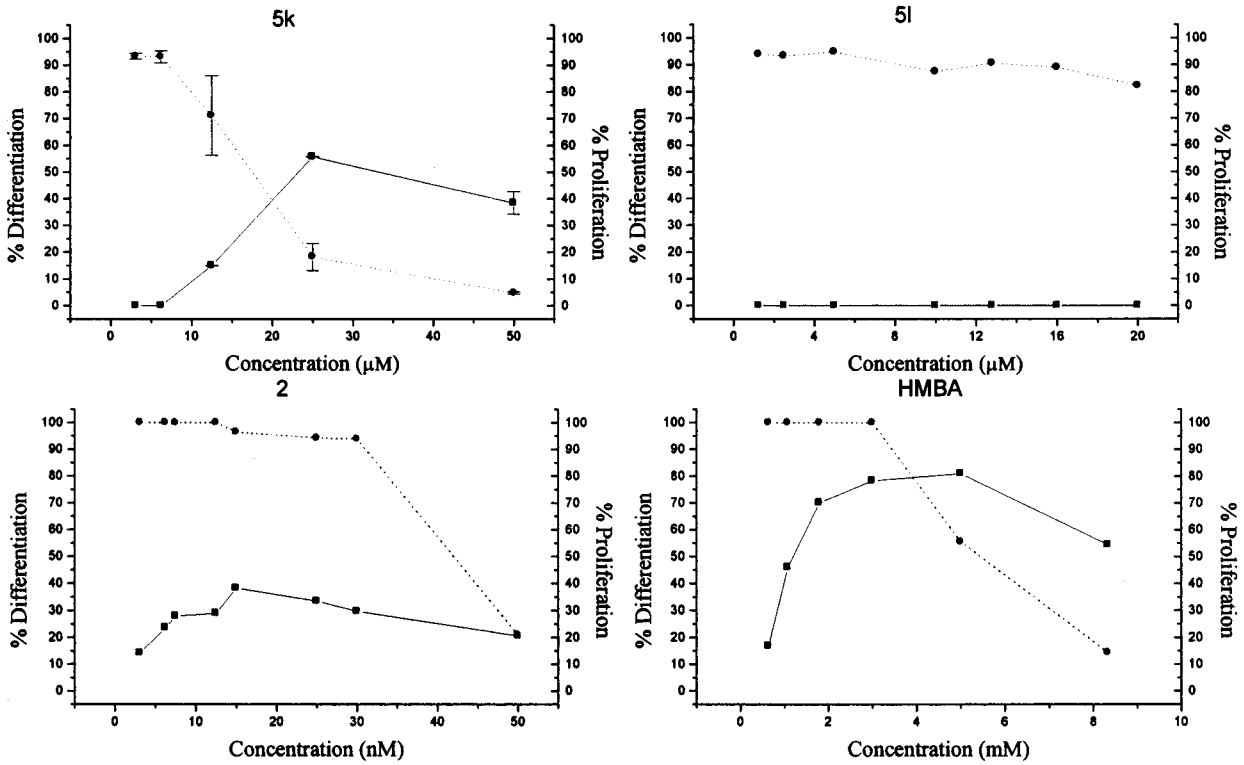


Figure 1. Induction of differentiation in Friend leukemic cells by amide analogues **5**. Squares and straight lines represent % differentiation, dots and dotted lines % proliferation.

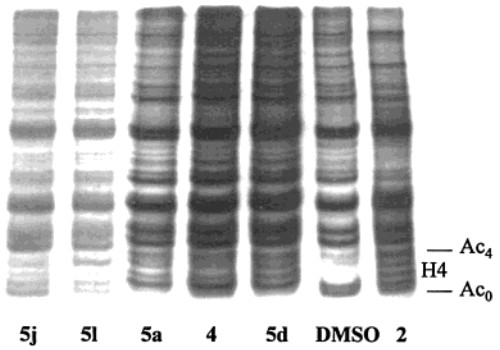


Figure 2. Hyperacetylation in Friend leukemic cells by inhibitors of HD. MEL cells were incubated for 6 h with **2** (330 nM), **4** (20 μM), **5a** (20 μM), **5d** (10 μM), **5j** (100 μM), and **5l** (100 μM). Histones were subsequently isolated and analyzed by AUT gels as described in the Experimental Section. DMSO, solvent control (2 μg/mL); H4, histone H4; Ac_n, number of acetylated lysine residues.

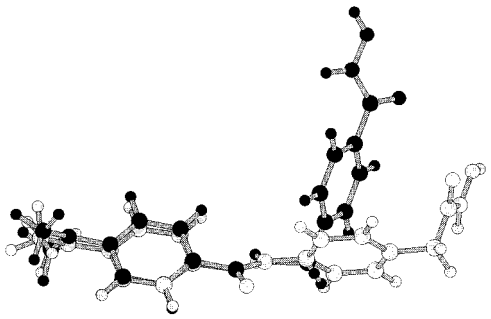


Figure 3. Superposition of 3D-structures of **5f** and **5g**. Force-field minimizations (MM^+) were performed as indicated in the Experimental Section using Hyperchem. **5f** is represented by white balls and **5g** by black balls.

ence can be explained by the three-dimensional structure of the latter two, although a three-dimensional structure of trichostatin within the enzyme is still not available. (Please see Note Added in Proof.) Due to the aromatic rings and amide bonds, **5f** and **5g** have only one methylene group which allows for greater flexibility within the molecule. As this methylene group is located in different parts of the molecules, the resulting structures have different orientations of the hydroxamate functionality relative to the benzamide moiety. This is visualized by the force-field-minimized structures presented in Figure 3. It is interesting to see that all modifications in the benzamide moiety lead to compounds more potent than the unsubstituted parent compound **5i** although the substituents cover different ranges of lipophilicity and σ-constants. We do not have an explanation for this generally increased affinity at this point.

We have demonstrated that simple analogues of trichostatin A **(2)** are able to induce histone hyperacetylation and differentiation in a leukemic cell line, therefore showing promising potential for the chemoprevention and treatment of malignant disease. This is especially supported by the fact that **2** is able to increase retinoid-mediated differentiation and even overcome retinoid resistance in cell culture[6,7] and in mice.[19] Furthermore, phenyl butyrate, an inhibitor of HD and inducer of terminal cell differentiation in the millimolar range,[20] was able to restore retinoid sensitivity in a patient with a highly resistant acute promyelocytic leukemia which resulted in complete remission and elimination of residual disease.[21]

Of the compounds tested only the hydroxamic acids were active in either assay. A potent carboxylic acid inhibitor of HD that promises oral bioavailability and

lower side effects remains yet to be found. This big difference in activity between the hydroxamates and the carboxylates can hardly be accounted merely to hydrogen bonding to the target protein and suggests the participation of a metal atom, possibly zinc, in the cleavage of the acetamide bond at the active site. Similar big differences in the activity between carboxylic and hydroxamic acids are known from the class of the matrix metalloprotease (MMP) inhibitors which are zinc-dependent enzymes.[22] Additional support for a zinc (or metal) hypothesis is coming from MS-275-like HD inhibitors. SAR studies showed the indispensability of an amino or hydroxy substituent in the 2′-position in the anilide.[13] Again this may point to possible chelation effects. Whereas in this case hydrogen bonding to the target is a possible alternative, this seems much less likely with our structures. (Please see Note Added in Proof.) Generally only compounds that inhibited HD enzymatic activity were inducers of cell differentiation. So in vitro assays for HD activity are able to select interesting compounds in primary screenings toward new anticancer agents, and recent developments in that field should be valuable tools for that goal.[23,24] However not all compounds with HD inhibitory activity induced differentiation in Friend cells. The reasons for these findings are currently not known. Similar behavior has been observed among some analogues of SAHA.[25]

We have presented a series of novel inhibitors of HD, some of which were shown to induce hyperacetylation in leukemic cells which correlated with their ability to induce terminal cell differentiation. On the basis of these characteristics, these compounds can be considered valuable mechanistic tools and a good starting point for further SAR studies to improve this therapeutically very promising class of compounds. The compounds that inhibit HD in vitro and in cell culture but fail to induce hyperacetylation might also be of interest from a mechanistic point of view. Maybe they interfere with a downstream signaling step that is crucial for mediating the effects caused by histone hyperacetylation.

Experimental Section

Chemical reagents were purchased from Aldrich or Fluka and used without further purification. Melting points are uncorrected. Elemental analysis was performed on a Foss-Heraeus CHN-O-Rapid. IR spectra were recorded on a Shimadzu 470 or a Biorad FTS 135 in KBr unless stated otherwise. ^{1}H NMR was done on a Varian Gemini 200 (200 MHz) and ^{13}C NMR on the same instrument (50.29 MHz). MS spectrometry was done on a Finnigan MAT 312 (ESI). Flash chromatography was performed using silica gel 60, 230−400 mesh (Merck). Dichloromethane was dried over molecular sieves (3 Å). The Ω-amino acid methyl esters were prepared from the commercially available acids using methanol/thionyl chloride according to standard procedures. D-MEM, penicillin G sodium, and streptomycin sulfate were purchased from Life Technologies.

Method A: Amide Formation Using BOP-Cl. To a solution or suspension of the acid in dry CH_2Cl_2 (5 mL/mmol) was added triethylamine (TEA, 1 equiv) under nitrogen, and the mixture was stirred for 10 min. Then BOP-Cl (1.1 equiv), the Ω-amino acid methyl ester, respectively, hydroxylamine hydrochloride (1 equiv), and again TEA (3 equiv) were added. After stirring overnight most of the CH_2Cl_2 was removed under reduced pressure and ethyl acetate (100 mL) and 3% $NaHCO_3$ solution (50 mL) were added. The organic phase was separated and washed consecutively with water and satd brine (50 mL each). The organic layer was dried over Na_2SO_4 and the solvent was evaporated.

Method B: Amide Formation Using Acid Chlorides. A solution of an acid chloride (10 mmol) and TEA (2.79 mL, 2.03 g, 20 mmol) was added dropwise over 15 min to a solution or suspension of the 6-aminocaproic acid methyl ester hydrochloride (1.82 g, 10 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred 30 min at 5 °C and 3 h at room temperature. The organic phase was then washed with 2 M HCl, water, 3% $NaHCO_3$, and satd brine (100 mL of each). The organic layer was dried over Na_2SO_4 and the solvent was evaporated.

Method C: Ester Cleavage. The ester **6** was dissolved in THF (2 mL/mmol) and an aqueous solution of LiOH (1 M, 2 equiv) was added. The mixture was stirred for 4 h at room temperature. Either the reaction mixture was evaporated, dried in a desiccator overnight over P_2O_5, and used as such for the next coupling step (**Method C1**) or 0.5 M NaOH (10 mL) and ethyl acetate (50 mL) were added. The aqueous phase was then acidified with 2 M HCl (pH 3) (**Method C2**). In the case of method C1 the pure acid was isolated by precipitation with 2 M HCl from a solution of some of the lithium salt.

Method D: Hydrogenation. The *N*-benzyloxy precursor was dissolved in methanol (5−10 mL/mmol) and 10% palladium on charcoal (10% w/w) was added. The mixture was treated with hydrogen under atmospheric pressure for 6 h and was filtered subsequently. The product was evaporated, redissolved in methanol, and precipitated with diethyl ether.

Methyl 6-(4-Dimethylaminobenzoyl)aminocaproate (5a). The synthesis and the analytical data have been described.[26] It was synthesized from 4-dimethylaminobenzoic acid using methods A, C1, A, and D like **5b**.

Methyl 4-(4-Dimethylaminobenzoyl)aminobutyrate (6b). Synthesized by method A from 4-dimethylaminobenzoic acid (1.65 g, 10 mmol), TEA (1.39 mL, 1.01 g, 10 mmol), BOP-Cl (2.80 g, 11 mmol), methyl 4-aminobutyrate hydrochloride (1.54 g, 10 mmol), TEA (4.17 mL, 3.03 g, 30 mmol). The crude was chromatographed using ethyl acetate/hexane (2:1): yield 1.16 g (44%); mp 105 °C; IR 1735, 1620; ^{1}H NMR ($CDCl_3$) δ 7.71−7.66 and 6.68−6.64 (m, 2H), 6.42 (bs, 1H), 3.65 (s, 3H), 3.52−3.42 (m, 2H), 3.01 (s, 6H), 2.47−2.40 (m, 2H), 2.01−1.91 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.20, 167.51, 152.51, 128.37, 121.45, 111.15, 51.66, 40.10, 39.45, 31.80, 24.84; MS(ESI) *m*/*z* 264 (M^+).

4-(4-Dimethylaminobenzoyl)aminobutyric Acid (7b). Synthesized by method C1 from **6b** (1.06 mg, 4 mmol): mp 172 °C; IR 1707, 1615; ^{1}H NMR (DMSO-d_6) δ 8.15 (t, ^3J = 5.27 Hz, 1H), 7.73−7.68 and 6.69−6.65 (m, 2H), 3.27−3.17 (m, 2H), 2.94 (s, 6H), 2.28−2.21 (m, 2H), 1.78−1.68 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 174.23, 166.11, 151.97, 128.37, 121.34, 110.69, 39.63, 38.48, 31.41, 24.79; MS(ESI) *m*/*z* 312 (M^+).

***N*-Benzyloxy-4-(4-dimethylaminobenzoyl)aminobutyramide (8b).** Synthesized by method A from crude **7b**, TEA (0.69 mL, 505 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-benzylhydroxylamine hydrochloride (770 mg, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The crude was chromatographed using ethyl acetate/methanol (20:1, 1% diethylamine): yield 280 mg (20% from **6b**); mp 116 °C; IR 1673, 1616; ^{1}H NMR (DMSO-d_6) δ 11.00 (s, 1H), 8.10 (t, ^3J = 5.4 Hz, 1H), 7.72−7.68 (m, 2H), 7.39 (s, 5H), 6.70−6.65 (m, 2H), 4.76 (s, 2H), 3.25−3.16 (m, 2H), 2.94 (s, 6H), 2.03−1.95 (m, 2H), 1.75−1.68 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.22, 166.09, 151.98, 135.99, 128.62, 128.36, 128.17, 128.08, 121.31, 110.69, 76.75, 39.62, 38.55, 29.98, 25.24; MS(ESI) *m*/*z* 355 (M^+).

***N*-Hydroxy-4-(4-dimethylaminobenzoyl)aminobutyramide (5b).** Synthesized by method D from **8b** (200 mg, 0.56 mmol), 10% Pd on charcoal (20 mg): yield 100 mg (67%) of white powder; mp 169 °C; IR 1610; ^{1}H NMR (DMSO-d_6) δ 10.40 (s, 1H), 8.70 (s, 1H), 8.13 (t, ^3J = 5.45 Hz, 1H), 7.72−7.66 and 6.71−6.65 (m, 2H), 3.24−3.15 (m, 2H), 2.94 (s, 6H, NMe_2), 2.04−1.96 (m, 2H), 1.77−1.67 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 168.92, 166.06, 151.96, 128.36, 121.30, 110.69, 39.63, 30.02, 25.45; MS(ESI) *m*/*z* 265 (M^+). Anal. ($C_{14}H_{21}N_2O_3$) C, H, N.

Methyl 5-(4-Dimethylaminobenzoyl)aminovalerate (6c). Synthesized by method A from 4-dimethylaminobenzoic acid (1.65 g, 10 mmol), TEA (1.39 mL, 1.01 g, 10 mmol), BOP−Cl (2.80 g, 11 mmol), methyl 5-aminovalerate hydrochloride (1.68 g, 10 mmol), TEA (4.17 mL, 3.03 g, 30 mmol). The crude was chromatographed using ethyl acetate/hexane (2:1, 1% diethylamine): yield 1.28 g (46%); mp 79 °C; IR 1731, 1610; ^{1}H NMR ($CDCl_3$) δ 7.71−7.67 and 6.69−6.63 (m, 2H), 6.32 (bs, 1H), 3.66 (s, 3H), 3.47−3.38 (m, 2H), 3.00 (s, 6H), 2.40−2.33 (m, 2H), 1.73−1.62 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.02, 167.50, 152.45, 128.36, 121.65, 111.18, 51.51, 40.12, 39.35, 33.60, 29.28, 22.22; MS(ESI) *m*/*z* 278 (M^+).

5-(4-Dimethylaminobenzoyl)aminovaleric Acid (7c). Synthesized by method C1 from **6c** (1.11 g, 4 mmol): mp 138 °C; IR 1722, 1622; ^{1}H NMR (DMSO-d_6) δ 8.09 (t, ^3J = 5.5 Hz, 1H), 7.72−7.67 and 6.69−6.65 (m, 2H), 3.28−3.18 (m, 2H), 2.94 (s, 6H), 2.14−2.08 (m, 2H), 1.52−1.44 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 174.41, 165.99, 151.98, 128.39, 121.41, 110.74, 39.72, 33.40, 28.87, 22.05; MS(ESI) *m*/*z* 264 (M^+).

***N*-Benzyloxy-5-(4-dimethylaminobenzoyl)aminovaleramide (8c).** Synthesized by method A from crude **7c**, TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-benzylhydroxylamine hydrochloride (770 mg, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The crude was chromatographed using ethyl acetate/methanol (20:1, 1% diethylamine): yield 1.12 g (74% from **6c**); mp 151 °C; IR 1675, 1607; ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 1H), 8.09 (bs, 1H), 7.72−7.68 and 6.69−6.65 (m, 2H), 7.35 (s, 5H), 4.76 (s, 2H), 3.21−3.18 (m, 2H), 1.97−1.94 (m, 2H), 1.48−1.46 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 168.93, 165.65, 151.62, 135.70, 128.33, 128.03, 127.85, 127.54, 121.13, 110.38, 76.41, 39.33, 38.30, 31.64, 28.53, 22.18; MS(ESI) *m*/*z* 369 (M^+).

***N*-Hydroxy-5-(4-dimethylaminobenzoyl)aminovaleramide (5c).** Synthesized by method D from **8c** (260 mg, 0.71 mmol), 10% Pd on charcoal (30 mg): yield 160 mg (81%) of white powder; mp 148 °C; IR 1660, 1595; ^{1}H NMR (CD_3OD) δ 7.73−7.66 and 6.74−6.67 (m, 2H), 3.38−3.28 (m, 2H), 2.16−2.09 (m, 2H), 1.70−1.57 (m, 2H); ^{13}C NMR (CD_3OD) δ 172.74, 170.45, 154.25, 129.66, 122.21, 112.18, 40.26, 33.37, 30.09, 24.10; MS(ESI) *m*/*z* 279 (M^+). Anal. ($C_{15}H_{23}N_3O_3$) C, H, N.

Methyl 7-(4-Dimethylaminobenzoyl)aminoheptanoate (6d). Synthesized by method A from 4-dimethylaminobenzoic acid (1.65 g, 10 mmol), TEA (1.39 mL, 1.01 g, 10 mmol), BOP−Cl (2.80 g, 11 mmol), methyl 7-aminoheptanoate hydrochloride (1.96 g, 10 mmol), TEA (4.17 mL, 3.03 g, 30 mmol). The crude was chromatographed using ethyl acetate/hexane (1:1, 1% diethylamine): yield 1.64 g (56%); mp 68 °C; ^{1}H NMR ($CDCl_3$) δ 7.70−7.65 and 6.68−6.64 (m, 2H), 6.12 (bs, 1H), 3.66 (s, 3H), 3.46−3.36 (m, 2H), 3.01 (s, 6H), 2.35−2.27 (m, 2H), 1.76−1.53 and 1.40−1.32 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 174.17, 167.48, 152.49, 128.34, 121.83, 51.44, 40.15, 39.81, 34.03, 29.74, 28.86, 26.67, 24.87; MS(ESI) *m*/*z* 292 (M^+).

7-(4-Dimethylaminobenzoyl)aminoheptanoic Acid (7d). Synthesized by method C1 from **6d** (1.17 g, 4 mmol): mp 124 °C; IR 1724, 1616; ^{1}H NMR ($CDCl_3$) δ 7.69−7.65 and 6.69−6.62 (m, 2H), 6.16 (bs, 1H), 3.40−3.36 (m, 2H), 3.00 (s, 6H), 2.38−2.30 (m, 2H); 1.64−1.57 and 1.41−1.34 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 178.18, 167.94, 152.56, 128.41, 121.62, 111.25, 40.16, 39.88, 33.92, 29.68, 28.74, 26.61, 24.69; MS(ESI) *m*/*z* 292 (M^+).

***N*-Benzyloxy-7-(4-dimethylaminobenzoyl)aminoheptanamide (8d).** Synthesized by method A from crude **7d**, TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-benzylhydroxylamine hydrochloride (770 mg, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The crude was chromatographed using ethyl acetate/methanol (20:1, 1% diethylamine): yield 990 mg (62% from **6d**); mp 136 °C; IR 1674, 1619; ^{1}H NMR (CD_3OD) δ 7.72−7.67 (m, 2H), 7.40−7.31 (m, 5H); 6.73−6.68 (m, 2H), 4.86 (s, 2H), 3.35−3.28 (m, 2H), 2.99 (s, 6H), 2.07−2.00 (m, 2H), 1.61−1.51 and 1.34−1.28 (m, 4H); ^{13}C NMR (CD_3OD) δ 172.89, 170.40, 154.19, 130.30, 129.64, 129.44, 122.22, 112.15, 78.91, 40.75, 40.27, 33.67, 30.51, 29.75, 27.67, 26.52; MS(ESI) *m*/*z* 397 (M^+).

***N*-Hydroxy-7-(4-dimethylaminobenzoyl)aminoheptanamide (5d).** Synthesized by method D from **8d** (600 mg, 1.51 mmol), 10% Pd on charcoal (60 mg): yield 360 mg (78%) of white powder; mp 161 °C; IR 1665, 1595; ^{1}H NMR (DMSO-d_6) δ 10.35 (s, 1H), 8.68 (s, 1H); 8.08−8.06 (m, 1H), 7.71−7.66 and 6.69−6.65 (m, 2H), 3.23−3.13 (m, 2H), 2.93 (s, 6H), 1.96−1.88 (m, 2H), 1.52−1.40 (m, 4H), 1.24 (bs, 4H); ^{13}C NMR (DMSO-d_6) δ 168.99, 165.87, 151.78, 128.20, 121.18, 110.54, 39.50, 39.06, 32.02, 29.02, 28.15, 26.02, 24.88; MS(ESI) *m*/*z* 307 (M^+). Anal. ($C_{16}H_{25}N_3O_3$) C, H, N.

Methyl 8-(4-Dimethylaminobenzoyl)aminooctanoate (6e). Synthesized by method A from 4-dimethylaminobenzoic acid (1.65 g, 10 mmol), TEA (1.39 mL, 1.01 g, 10 mmol), BOP−Cl (2.80 g, 11 mmol), methyl 8-aminooctanoate hydrochloride (2.10 g, 10 mmol), TEA (4.17 mL, 3.03 g, 30 mmol). The crude was chromatographed using ethyl acetate/hexane (2:1, 1% diethylamine): yield 2.25 g (70%); mp 62 °C; IR 1738, 1618; ^{1}H NMR ($CDCl_3$) δ 7.71−7.67 and 6.67−6.63 (m, 2H), 6.21 (bs, 1H), 3.66 (s, 3H), 3.45−3.35 (m, 2H), 2.33−2.26 (m, 2H), 1.65−1.55 (m, 4H), 1.34−1.32 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 174.25, 167.45, 152.40, 128.33, 121.75, 111.12, 51.44, 40.14, 39.87, 34.07, 29.83, 29.06, 28.98, 26.84, 24.87; MS(ESI) *m*/*z* 320 (M^+).

8-(4-Dimethylaminobenzoyl)aminooctanoic Acid (7e). Synthesized by method C1 from **6e** (1.28 g, 4 mmol): mp 154 °C; IR 1708, 1604; ^{1}H NMR (DMSO-d_6) δ 8.09−8.03 (m, 1H), 7.71−7.67 and 6.69−6.65 (m, 2H), 3.28−3.13 (m, 2H), 2.94 (s, 6H), 2.21−2.13 (m, 2H), 1.50−1.40 (m, 4H), 1.39−1.26 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 174.47, 165.96, 151.94, 128.38, 121.49, 110.73, 39.70, 38.97, 33.73, 29.30, 28.53, 28.51, 26.39, 24.48; MS(ESI) *m*/*z* 306 (M^+).

***N*-Benzyloxy-8-(4-dimethylaminobenzoyl)aminooctanamide (8e).** Synthesized by method A from crude **7e**, TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-benzylhydroxylamine hydrochloride (770 mg, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The product precipitated upon evaporation of the organic layer and was collected by filtration: yield 770 mg (47% from **6e**); mp 134 °C; IR 1670, 1607; ^{1}H NMR (CD_3OD) δ 7.72−7.67 (m, 2H), 7.40−7.31 (m, 5H), 6.73−6.68 (m, 2H), 4.86 (s, 2H), 3.35−3.28 (m, 2H), 2.99 (s, 6H), 2.07−2.00 (m, 2H), 1.61−1.51 (m, 4H), 1.34−1.28 (m, 4H); ^{13}C NMR (CD_3OD) δ 167.72, 152.53, 129.16, 128.56, 128.40, 121.65, 112.49, 111.24, 78.23, 40.16, 39.63, 32.83, 29.79, 28.77, 28.44, 26.49, 24.95; MS(ESI) *m*/*z* 411 (M^+).

***N*-Hydroxy-8-(4-dimethylaminobenzoyl)aminooctanamide (5e).** Synthesized by method D from **8e** (300 mg, 0.73 mmol), 10% Pd on charcoal (30 mg): yield 140 mg (60%) of white powder; mp 158 °C; IR 1610, 1555; ^{1}H NMR (DMSO-d_6) δ 10.33 (s, 1H), 8.66 (s, 1H), 8.08−8.06 (m, 1H), 7.71−7.67 and 6.69−6.65 (m, 2H), 3.23−3.14 (m, 2H), 2.93 (s, 6H), 1.95−1.88 (m, 2H), 1.50−1.43 (m, 4H) 1.41−1.25 (m, 6H); ^{13}C (DMSO-d_6) δ 169.08, 165.95, 151.94, 128.38, 121.46, 110.72, 39.70, 38.95, 32.22, 29.31, 28.53, 28.47, 26.40, 25.05; MS(ESI) *m*/*z* 321(M^+). Anal. ($C_{17}H_{27}N_3O_3$) C, H, N.

Methyl 4-(4-Dimethylaminobenzoyl)aminophenylacetate (6f). Synthesized by method A from 4-dimethylaminobenzoic acid (1.65 g, 10 mmol), TEA (1.39 mL, 1.01 g, 10 mmol), BOP−Cl (2.80 g, 11 mmol), methyl 4-aminophenylacetate hydrochloride (3.49 g, 10 mmol), TEA (4.17 mL, 3.03 g, 30 mmol). The resulting suspension was extracted four times with ethyl acetate (100 mL each) and the combined organic extracts were evaporated after adding 1 g of silica. The powder was subjected to chromatography using ethyl acetate/hexane (2:1, 1% diethylamine) and the main fraction was evaporated. The resulting product was recrystallized form acetone/hexane: yield 1.68 g (90%); mp 172 °C; IR 1729, 1646; ^{1}H NMR (acetone-d_6) δ 9.24 (s, 1H), 7.92−7.87, 7.82−7.77, 7.26−7.22 and 6.78−6.73 (m, 2H), 3.64 (s, 3H), 3.61 (s, 2H), 3.03 (s, 6H); ^{13}C NMR (acetone-d_6) δ 172.40, 166.08, 153.71, 139.79, 130.24, 130.04, 129.76, 122.81, 120.82, 120.74, 111.82, 51.92, 40.81, 40.15; MS(ESI) *m*/*z* 312 (M^+).

4-(4-Dimethylaminobenzoyl)aminophenylacetic Acid (7f). Synthesized by method C1 from **6f** (1.25 g, 4 mmol): mp 227 °C; IR 1716, 1611; ^{1}H NMR (DMSO-d_6) δ 12.2 (bs, 1H), 9.84 (s, 1H), 7,88−7.83, 7.76−7.67, 7.21−7.16 and 6.77−6.72 (m, 2H), 3.51 (s, 2H), 2.98 (s, 6H); MS(ESI) *m*/*z* 298 (M^+).

***N*-Benzyloxy-4-(4-dimethylaminobenzoyl)aminophenylacetamide (8f).** Synthesized by method A from crude **7f**, TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-benzylhydroxylamine hydrochloride (770 mg, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The organic layer was evaporated after adding 500 mg of silica. The powder was subjected to chromatography using ethyl acetate (1% diethylamine): yield 790 mg (49% from **6f**); mp 179 °C; IR 1681, 1629; ^{1}H NMR (DMSO-d_6) δ 11.24 (s, 1H), 9.85 (s, 1H), 7.89−7.84, 7.71−7.67, 7.19−7.15 and 6.76−6.72 (m, 2H), 7.36 (s, 5H), 4.78 (s, 2H), 2.98 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 167.20, 164.93, 152.20, 138.03, 135.79, 129.83, 128.89, 128.74, 128.63, 128.08, 120.96, 120.00, 110.59, 76.60, 39.65, 39.49; MS(ESI) *m*/*z* 403 (M^+).

***N*-Hydroxy-4-(4-dimethylaminobenzoyl)aminophenylacetamide (5f).** Synthesized by method D from **8f** (190 mg, 0.47 mmol), 10% Pd on charcoal (25 mg); 30 mL of glacial acetic acid was added to dissolve the starting material. During the course of the reaction a white precipitate was formed. After 16 h at room temperature 30 mL of DMF was added to dissolve that precipitate. The charcoal was filtered off and the mixture was poured into 500 mL of water. After 4 days at 5 °C colorless crystals were collected by filtration: yield 60 mg (41%); mp 216 °C; IR 1637, 1615; ^{1}H NMR (DMSO-d_6) δ 10.62 (s, 1H), 9.82 (s, 1H), 8.81 (s, 1H), 7.87−7.84, 7.68−7.64, 7.20−7.16 and 6.76−6.72 (m, 2H), 3.25 (s, 2H), 2.99 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 167.17, 165.06, 152.34, 138.01, 130.52, 128.99, 128.82, 121.03, 110.71, 39.61 (1 resonance obscured); MS(ESI) *m*/*z* 313 (M^+). Anal. ($C_{17}H_{19}N_3O_3$) C, H, N.

Methyl 4-(4-Dimethylaminobenzoyl)aminomethylbenzoate (6g). Synthesized by method A from 4-dimethylaminobenzoic acid (1.65 g, 10 mmol), TEA (1.39 mL, 1.01 g, 10 mmol), BOP−Cl (2.80 g, 11 mmol), methyl 4-aminomethylbenzoate hydrochloride (3.49 g, 10 mmol), TEA (4.17 mL, 3.03 g, 30 mmol); 1 g of silica was added to the organic phase and after evaporation the crude was chromatographed using ethyl acetate/hexane (1:1, 1% diethylamine): yield 1.31 g (47%); mp 159 °C; IR 1718, 1626; ^{1}H NMR ($CDCl_3$) δ 7.98−7.94, 7.75−7.70, 7.39−7.35, 6.68−6.62 (m, 2H), 4.65 (d, ^3J = 5.9 Hz, 2H), 3.89 (s, 3H), 3.00 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 167.49, 166.94, 152.71, 144.41, 129.98, 129.24, 128.60, 127.56, 120.97, 111.18, 52.06, 43.55, 40.11; MS(ESI) *m*/*z* 312 (M^+).

4-(4-Dimethylaminobenzoyl)aminomethylbenzoic Acid (7g). Synthesized by method C1 from **6g** (1.25 g, 4 mmol): mp 238 °C; IR 1685, 1630; ^{1}H NMR (DMSO-d_6) δ 8.77 (t, ^3J = 6.4 Hz, 1H), 7.91−7.87, 7.79−7.75, 7.41−7.37 and 6.72−6.68 (m, 2H), 4.51 (d, ^3J = 6.4 Hz, 2H), 2.95 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 167.16, 166.17, 152.15, 145.28, 129.21, 128.54, 127.01, 120.79, 110.75, 42.24, 39.61; MS(ESI) *m*/*z* 298 (M^+).

***N*-Allyloxy-4-(4-dimethylaminobenzoyl)aminomethylbenzamide.** Synthesized by method A from crude **7g**, TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-allylhydroxylamine hydrochloride (550 mg, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The compound precipitated upon evaporation of the ethyl acetate layer and was collected by filtration: yield 310 mg (22% from **6g**); ^{1}H NMR (DMSO-d_6) δ 11.61 (s, 1H), 8.73 (bs, 1H), 7.79−7.74, 7.71−7.67, 7.38−7.31 and 6.72−6.68 (m, 2H), 6.00−5.92 (m, 1H), 5.37−5.22 (m, 2H), 4.49−4.38 (m, 2H), 4.36 (s, 2H), 2.95 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 166.15, 164.21, 152.14, 144.00, 133.10, 130.64, 128.52, 126.97, 120.83, 119.03, 110.76, 75.94, 42.19, 39.63; MS(ESI) *m*/*z* 353 (M^+).

***N*-*tert*-Butyldimethylsilyloxy-4-(4-dimethylaminobenzoyl)aminomethylbenzamide (8g).** Synthesized by method A from crude **7g**, TEA (0.69 mL, 550 g, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-*tert*-butyldimethylsilyl hydroxylamine (740 g, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The crude was chromatographed using ethyl acetate/methanol (20:1, 1% diethylamine): yield 130 mg (8%); mp 154 °C dec; IR 1610; ^{1}H NMR (CD_3OD) δ 7.78−7.72, 7.70−7.66, 7.44−7.40 and 6.75−6.71 (m, 2H), 4.59 (s, 2H), 3.01 (s, 6H), 1.01 (s, 9H), 0.23 (s, 6H); ^{13}C NMR (CD_3OD) δ 170.44, 154.43, 144.99, 132.29, 129.82, 128.52, 128.39, 128.28, 121.79, 112.21, 44.05, 40.22, 26.32, 19.14, −5.37.

***N*-Hydroxy-4-(4-dimethylaminobenzoyl)aminomethylbenzamide (5g).** TBAF·3H_2O (158 mg, 0.5 mmol) was added to a solution of **8g** (80 mg, 0.19 mmol) in 5 mL of dry THF. After stirring at room temperature for 4 h the mixture was evaporated and the crude was redissolved in acetone. The product was precipitated by adding water: yield 20 mg (34%) of white powder; mp 207 °C; ^{1}H NMR (DMSO-d_6) δ 8.72 (t, ^3J = 5.7 Hz, 1H), 7.78−7.73, 7.71−7.67, 7.36−7.31 and 6.72−6.68 (m, 2H), 4.46 (d, ^3J = 5.7 Hz, 2H), 2.95 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 166.13, 164.03, 152.14, 143.39, 131.14, 128.50, 126.88, 126.74, 120.84, 110.76, 42.16, 39.62; MS(ESI) *m*/*z* 313 (M^+). Anal. ($C_{17}H_{19}N_3O_3$) C, H, N.

Methyl 6-(4-Methoxybenzoyl)aminocaproate (6h). Synthesized by method B from anisoyl chloride (1.71 g, 10 mmol). The crude was chromatographed using ethyl acetate/hexane (3:1): yield 2.63 g (94%); mp 52 °C; IR 1739, 1636; ^{1}H NMR ($CDCl_3$) δ 7.78−7.71 and 6.94−6.88 (m, 2H), 6.32 (bs, 1H), 3.84 (s, 3H), 3.66 (s, 3H), 3.48−3.38 (m, 2H), 2.36−2.29 (m, 2H), 1.74−1.55 (m, 4H), 1.47−1.35 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.08, 167.10, 162.15, 128.71, 127.21, 113.77, 55.43, 51.50, 39.75, 33.91, 30.00, 26.48, 24.52; MS(ESI) *m*/*z* 279 (M^+).

6-(4-Methoxybenzoyl)aminocaproic Acid (7h). Synthesized by method C2 from **6h** (3.30 g, 11.83 mmol). The compound precipitated upon acidification of the aqueous layer: yield 2.26 g (72%); mp 102 °C; IR 1700, 1635; ^{1}H NMR (DMSO-d_6) δ 11.7 (bs, 1H), 8.28 (t, ^3J = 5.2 Hz, 1H), 7.82−7.78 and 6.99−6.94 (m, 2H), 3.78 (s, 3H), 3.25−3.16 (m, 2H), 2.23−2.16 (m, 2H), 1.51−1.47 (m, 4H), 1.35−1.23 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 174.29, 165.50, 161.29, 128.79, 126.94, 113.31, 55.20, 38.50, 33.55, 28.84, 25.98, 24.20; MS(ESI) *m*/*z* 265 (M^+).

***N*-Benzyloxy-6-(4-methoxybenzoyl)aminocapramide (8h).** Synthesized by method A from **7h** (2.20 g, 8.3 mmol), TEA (1.39 mL, 1.01 g, 10 mmol), BOP−Cl (2.80 g, 11 mmol), *O*-benzylhydroxylamine hydrochloride (1.54 g, 10 mmol), TEA (4.17 mL, 3.03 g, 30 mmol). The compound precipitated during the evaporation of the organic layer: yield 1.57 g (50%); mp 93 °C; IR 1677, 1634; ^{1}H NMR (DMSO-d_6) δ 10.94 (s, 1H), 8.27 (t, ^3J = 5.4 Hz, 1H), 7.82−7.78 and 6.97−6.93 (m, 2H), 4.75 (s, 2H), 3.78 (s, 3H), 3.25−3.16 (m, 2H), 1.98−1.91 (m, 2H), 1.58−1.41 (m, 4H), 1.32−1.17 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.30, 165.52, 161.33, 136.04, 128.85, 128.70, 128.23 128.14, 126.90, 113.34, 107.07, 76.73, 55.26, 38.95, 32.17, 28.91, 25.96, 24.69; MS(ESI) *m*/*z* 370 (M^+).

***N*-Hydroxy-6-(4-methoxybenzoyl)aminocapramide (5h).** Synthesized by method D from **8h** (350 mg, 0.95 mmol), 10% Pd on charcoal (40 mg): yield 210 mg (79%) of white powder; mp 142 °C; IR 1680, 1610; ^{1}H NMR (DMSO-d_6) δ 10.34 (s, 1H), 8.67 (bs, 1H), 8.29 (t, ^3J = 5.4 Hz, 1H), 7.82−7.78 and 6.98−6.94 (m, 2H), 3.78 (s, 3H), 3.25−3.16 (m, 2H), 1.97−1.90 (m, 2H), 1.52−1.42 (m, 4H), 1.31−1.20 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.08, 165.55, 161.35, 128.83, 126.94, 113.34, 113.34, 55.26, 38.99, 32.20, 28.91, 26.09, 24.85; MS(ESI) *m*/*z* 280 (M^+). Anal. ($C_{14}H_{20}N_2O_4$) C, H, N.

Methyl 6-(4-Benzoyl)aminocaproate (6i). Synthesized by method A from benzoic acid (1.22 g, 10 mmol), TEA (1.39 mL, 1.01 g, 10 mmol), BOP−Cl (2.80 g, 11 mmol), methyl 6-aminocaproate hydrochloride (1.82 g, 10 mmol), TEA (4.17 mL, 3.03 g, 30 mmol). The crude was chromatographed using ethyl acetate/hexane (1:1): yield 1.25 g (50%) of viscous oil; IR (neat) 1738, 1642; ^{1}H NMR ($CDCl_3$) δ 7.82−7.76 and 7.51−7.30 (m, 3H), 6.71 (bs, 1H), 3.65 (s, 3H), 3.47−3.37 (m, 2H), 3.78 (s, 3H), 2.35−2.27 (m, 2H), 1.72−1.54 (m, 4H), 1.45−1.26 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.14, 167.58, 134.84, 131.36, 128.58, 126.91, 51.55, 39.80, 33.90, 29.33, 26.44, 24.49; MS(ESI) *m*/*z* 249 (M^+).

6-(4-Benzoyl)aminocaproic Acid (7i). Synthesized by method C2 from **6i** (1.12 g, 4.5 mmol). The acid precipitated upon acidification of the aqueous layer: yield 730 mg (69%); mp 80 °C; IR 1700, 1631; ^{1}H NMR ($CDCl_3$) δ 7.78−7.74 (m, 2H), 7.53−7.36 (m, 3H), 6.37 (bs, 1H), 3.66−3.40 (m, 2H), 2.40−2.33 (m, 2H), 1.75−1.59 (m, 4H), 1.49−1.34 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 178.44, 167.87, 134.79, 131.45, 128.63, 126.96, 39.90, 33.81, 29.33, 26.40, 24.33; MS(ESI) *m*/*z* 235 (M^+).

***N*-Benzyloxy-6-(4-benzoyl)aminocapramide (8i).** Synthesized by method A from **7i** (710 mg, 3 mmol), TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-benzylhydroxylamine hydrochloride (770 mg, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The crude was chromatographed using ethyl acetate: yield 710 mg (70%); mp 110 °C; IR 1653, 1631; ^{1}H NMR ($CDCl_3$) δ 9.02 (bs, 1H), 7.79−7.74 (m, 2H), 7.48−7.27 (m, 8H), 6.57 (bs, 1H), 4.86 (s, 2H), 3.45−3.35 (m, 2H), 2.06 (bs, 2H), 1.68−1.51 (m, 4H), 1.38−1.26 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 171.07, 167.25, 131.42, 129.22, 128.78, 128.65, 128.60, 127.00, 78.37, 39.76, 32.77, 29.22, 26.32, 24.53; MS-(ESI) *m*/*z* 233 (M^+ − OBn).

***N*-Hydroxy-6-(4-benzoyl)aminocapramide (5i).** Synthesized by method D from **8i** (250 mg, 0.74 mmol), 10% Pd on charcoal (30 mg): yield 110 mg (60%) of white powder; mp 122 °C; IR 1635, 1530; ^{1}H NMR (DMSO-d_6) δ 10.35 (bs, 1H), 8.68 (s, 1H), 8.46−8.44 (m, 1H), 7.83−7.79 (m, 2H), 7.50−7.40 (m, 3H), 3.27−3.17 (m, 2H), 1.97−1.90 (m, 2H), 1.53−1.42 (m, 4H), 1.32−1.23 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.17, 166.17, 134.71, 131.00, 128.23, 127.10, 39.10, 32.26, 28.87, 26.13, 24.91; MS(ESI) *m*/*z* 250 (M^+). Anal. ($C_{13}H_{18}N_2O_3$) C, H, N.

Methyl 6-(4-Chlorobenzoyl)aminocaproate (6j). Synthesized by method B from 4-chlorobenzoyl chloride (1.77 g, 10 mmol). The ester precipitated during the evaporation of the organic layer and was collected by filtration: yield 2.33 g (94%); mp 67 °C; IR 1731, 1630; ^{1}H NMR ($CDCl_3$) δ 7.70−7.66 and 7.34−7.28 (m, 2H), 6.85 (bs, 1H), 3.61 (s, 3H), 3.40−3.30 (m, 2H), 2.30−2.23 (m, 2H), 1.63−1.52 (m, 4H,), 1.39−1.32 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.02, 166.56, 137,40, 133.12, 128.60, 128.41, 51.44, 39.80, 33.76, 29.09, 26.33, 24.24; MS(ESI) *m*/*z* 250/248 (M^+).

6-(4-Chlorobenzoyl)aminocaproic Acid (7j). Synthesized by method C2 from **6j** (2.10 g, 8.4 mmol). The acid precipitated upon acidification of the aqueous phase: yield 1.50 g (57%); mp 125 °C; IR 1737, 1618; ^{1}H NMR (CD_3OD) δ 7.82−7.75 and 7.48−7.43 (m, 2H), 3.39−3.32 (m, 2H), 2.33−2.27 (m, 2H), 1.69−1.56 (m, 4H), 1.47−1.36 (m, 2H); ^{13}C NMR (CD_3-OD) δ 177.42, 169.03, 138.57, 134.56, 129.91, 129.66, 40.89, 34.82, 30.10, 27.55, 25.74; MS(ESI) *m*/*z* 271/269 (M^+).

***N*-Trityloxy-6-(4-chlorobenzoyl)aminocapramide (8j).** Synthesized by method A from **7j** (1.35 g, 5 mmol), TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.05 mmol), *O*-tritylhydroxylamine (1.38 g, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The compound precipitated upon evaporation of the organic layer: yield 1.50 g (57%); mp 159 °C; IR 1683, 1636; NMR spectra of the *O*-trityl compounds were only partially readable due to excessive line broadening and weak signal intensity and were therefore not evaluated; MS(ESI) *m*/*z* 243 (Trt^+).

***N*-Hydroxy-6-(4-chlorobenzoyl)aminocapramide (5j).** **8j** (1.12 g, 2 mmol) was dissolved in a mixture of TFA (10 mL) and CH_2Cl_2 (10 mL). The mixture was then treated dropwise with Et_3SiH from a syringe until the color changed from red to pale yellow. After 2 h of stirring at room temperature 30 mL of methanol was added and the mixture was evaporated. The crude was treated with diethyl ether and the resulting precipitate was collected by filtration: yield 180 mg (32%) of white powder; mp 162 °C; IR 1679, 1608; ^{1}H NMR (DMSO-d_6) δ 10.34 (s, 1H), 8.67 (s, 1H), 8.52 (t, ^3J = 5.4 Hz, 1H), 7.86−7.82 and 7.53−7.49 (m, 2H), 3.26−3.14 (m, 2H) 1.97−1.90 (m, 2H), 1.57−1.42 (m, 4H), 1.35−1.20 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.10, 165.04, 135.80, 133.40, 129.05, 128.28, 39.17, 32.22, 28.78, 26.09, 24.89; MS(ESI) *m*/*z* 254/252 (M^+ − NHOH).

Methyl 6-(4-Nitrobenzoyl)aminocaproate (6k). The compound was synthesized using method B from 4-nitrobenzoyl chloride (1.88 g, 10 mmol). The crude was recrystallized from dichloromethane/hexane: yield 2.23 (76%); mp 82 °C; IR 1730, 1635; ^{1}H NMR ($CDCl_3$) 8.30−8.23 and 8.01−7.94 (m, 2H), 6.84 (bs, 1H), 3.67 (s, 3H), 3.53−3.45 (m, 2 H), 2.37−2.31 (m, 2H), 1.75−1.59 (m, 4H), 1.49−1.37 (m, 2H); MS(ESI) *m*/*z* 294 (M^+).

6-(4-Nitrobenzoyl)aminocaproic Acid (7k). Synthesized by method C2 from **6k** (2.20 g, 7.48 mmol). The acid precipitated upon acidification of the aqueous layer: yield 1.42 g (68%); mp 148 °C; IR 1738, 1647; ^{1}H NMR (DMSO-d_6) δ 12.38 (bs, 1H), 8,79 (t, ^3J = 5.57 Hz, 1H), 8.29 (m, 2H, Ar−H), 8.04 (m, 2 H, Ar−H), 3.31−3.21 (m, 2H), 2.24−2.17 (m, 2H), 1.55−1.45 (m, 4H), 1.37−1.25 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 174.27, 164.39, 148.84, 140.29, 128.53, 123.33, 39.20, 33.52, 28.53, 25.92, 24.14; MS(ESI) *m*/*z* 280 (M^+).

***N*-Trityloxy-6-(4-nitrobenzoyl)aminocapramide (8k).** Synthesized by method A from **7k** (1.30 g, 4.64 mmol), TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-tritylhydroxylamine (1.38 g, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The product precipitated during evaporation of the organic layer: yield 1.62 g (65%); mp 205 °C; IR 1670, 1635; NMR spectra of the *O*-trityl compounds were only partially readable due to excessive line broadening and weak signal intensity and were therefore not evaluated; MS(ESI) *m*/*z* 243 (Trt^+).

***N*-Hydroxy-6-(4-nitrobenzoyl)aminocapramide (5k). 8k** (300 mg, 0.54 mmol) was dissolved in a mixture of TFA (7 mL) and CH_2Cl_2 (7 mL). The mixture was then treated dropwise with Et_3SiH from a syringe until the color changed from red to pale yellow. After 2 h of stirring at room temperature 30 mL of methanol was added and the mixture was evaporated. The crude was treated with diethyl ether and the product was precipitated by adding hexane: yield 130 mg (82%) of pale yellow powder; mp 139 °C; IR 1655, 1635; ^{1}H NMR (DMSO-d_6) δ 10.37 (bs, 1H), 8.81−8.75, 8.31−8.26 and 8.05−8.01 (m, 2H), 3.29−3.20 (m, 2H), 1.97−1.90 (m, 2H), 1.54−1.47 (m, 4H), 1.33−1.26 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.38, 164.80, 149.05, 140.45, 128.75, 123.61, 53.26, 28.75, 26.17, 24.97; MS-(ESI) *m*/*z* 263 (M^+ − NHOH). Anal. ($C_{12}H_{17}N_3O_5$) C, H, N.

Methyl 6-(4-Biphenylylcarbonyl)aminocaproate (6l). Synthesized using method B from biphenylcarbonyl chloride (2.20 g, 10 mmol). The crude was chromatographed using ethyl acetate/hexane (2:1). Impure fractions were reseparated using the same solvent: combined yield 2.30 g (71%); mp 114 °C; IR 1733, 1630; ^{1}H NMR ($CDCl_3$) δ 7.87−7.82 (m, 2H), 7.65−7.58 (m, 4H), 7.50−7.37 (m, 3H), 6.44 (bs, 1H), 3.66 (s, 3H), 3.51−3.43 (m, 2 H), 2.36−2.30 (m, 2H), 1.75−1.58 (m, 4H), 1.48−1.37 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.09, 167.29, 144.25, 140.17, 133.57, 128.98, 128.02, 127.49, 127.27, 51.53, 39.87, 33.94, 29.39, 26.51, 24.53; MS(ESI) *m*/*z* 325 (M^+).

6-(4-Biphenylylcarbonyl)aminocaproic Acid (7l). Synthesized by method C2 from **6l** (2.26 g, 6.96 mmol). The acid precipitated upon acidification of the aqueous layer: yield 1.74 g (80%); mp 169 °C; IR 1719, 1693, 1638; ^{1}H NMR (DMSO-d_6) δ 8.54−8.49 (m, 1H), 7.95−7.91 (m, 2H), 7.76−7.69 (m, 4H), 7.51−7.35 (m, 3H), 3.30−3.20 (m, 2H), 2.50−2.48 (m, 2H), 1.58−1.48 (m, 4H), 1.36−1.25 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 174.74, 165.63, 142.41, 139.17, 133.49, 128.87, 127.83, 127.70, 126.72, 126.30, 34.33, 28.78, 26.09, 24.47; MS(ESI) *m*/*z* 311 (M^+).

***N*-Benzyloxy-6-(4-biphenylylcarbonyl)aminocapramide (8l).** Synthesized by method A from **7l** (1.56 g, 5 mmol), TEA (0.69 mL, 550 mg, 5 mmol), BOP−Cl (1.40 g, 5.5 mmol), *O*-benzylhydroxylamine hydrochloride (770 mg, 5 mmol), TEA (2.08 mL, 1.01 g, 15 mmol). The aqueous suspension was extracted thrice with ethyl acetate (100 mL each). After drying over Na_2SO_4 the product precipitated during evaporation of the solvent and was collected by filtration: yield 1.29 g (62%); mp 150 °C; IR 1650, 1634; ^{1}H NMR (DMSO-d_6) δ 10.96 (bs, 1H), 8.50−8.48 (m, 1H), 7.95−7.90 (m, 2H), 7.75−7.68 (m, 4H), 7.52−7.34 (m, 3H), 7.35 (s, 5H), 4.75 (s, 2H), 3.27−3.24 (m, 2H), 1.96−1.92 (m, 2H), 1.56−1.48 (m, 4H), 1.30−1.26 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.30, 165.70, 142.48, 139.17, 136.02, 133.47, 128.94, 128.68, 128.21, 128.12, 127.92, 127.74, 126.77, 126.37, 76.73, 32.17, 28.80, 25.94, 24.67; MS(ESI) *m*/*z* 416 (M^+).

***N*-Hydroxy-6-(4-biphenylylcarbonyl)aminocapramide (5l).** Synthesized by method D from **8l** (500 mg, 1.20 mmol), 10% Pd on charcoal (50 mg). The product precipitated upon evaporation of the methanol and it was washed with diethyl ether: yield 180 mg (46%) of white powder; mp 187 °C; IR 1630, 1535; ^{1}H NMR (DMSO-d_6) δ 10.38 (bs, 1H), 8.71 (s, 1H), 8.54−8.48 (m, 1H), 7.95−7.91 (m, 2H), 7.76−7.69 (m, 4H9, 7.47−7.34 (m, 3H), 3.30−3.21 (m, 2H), 1.99−1.92 (m, 2H),

1.56−1.45 (m, 4H), 1.34−1.27 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 169.21, 165.86, 142.60, 139.25, 133.53, 129.05, 128.03, 127.85, 126.86, 126.48, 39.17, 32.29, 28.92, 26.16, 24.96; MS(ESI) *m/z* 326 (M^+). Anal. ($C_{19}H_{22}N_2O_3$) C, H, N.

Enzyme Inhibition. Radioactively labeled chicken core histones were used as the enzyme substrate according to established procedures.[27] The enzyme liberated tritiated acetic acid from the substrate which was quantitated by scintillation counting. IC_{50} values are results of triple determinations. 50 μL of maize enzyme (at 30 °C) was incubated (30 min) with 10 μL of total [^{3}H]acetate-prelabeled chicken reticulocyte histones (1 mg/mL). Reaction was stopped by addition of 36 μL of 1 M HCl/0.4 M acetate and 800 μL of ethyl acetate. After centrifugation (10000*g*, 5 min) an aliquot of 600 μL of the upper phase was counted for radioactivity in 3 mL of liquid scintillation cocktail. The compounds were tested in a starting concentration of 40 μM, and active substances were diluted further. **2** was used as the reference compound, and blank solvents were used as negative controls.

Induction of Differentiation. MEL DS19 cells (murine erythroleukemia cells) were maintained in D-MEM containing 100 units/mL penicillin G sodium and 100 μg/mL streptomycin sulfate supplemented with 10% fetal bovine serum (Greiner) at 37 °C in a 5% CO_2 atmosphere. To test compounds for potential to induce cell differentiation, log-phase cells with a population doubling time of 11−13 h were used. Serial dilutions of compounds were prepared in 24-well plates (Falcon) using 1 mL of D-MEM/well. If compounds were dissolved in DMSO, control wells contained the same amount of solvent (generally 2 μL/mL of medium). Subsequently, the cell suspension was added to the wells (1 mL/well, 8×10^4 cells/mL; final cell concentration 4×10^4 cells/well). After 72 h the experiment was evaluated. Cell numbers were counted using a Casy 1 TTC flow cytometer (Schärfe System). The proliferation of treated cells was expressed as percent proliferation in comparison with the solvent control.

Differentiated MEL cells accumulate hemoglobin. Therefore, the induction of cell differentiation was determined by benzidine staining according to the literature.[28] To 100 μL of cell suspension was added 10 μL of a 0.4% solution of benzidine in 12% acetic acid containing 2% H_2O_2. Within 5 min hemoglobin-containing cells stained blue. Benzidine-positive and -negative cells were counted under the microscope in a hemocytometer, and the percentage of positive cells was calculated. All compounds were first tested at 10 and 50 μM final concentration. According to activity/toxicity profile, a range of concentrations was chosen for a dose−response analysis. **2** and hexamethylenebisacetamide (HMBA), a known inducer of cell differentiation in MEL cells that is not a HD inhibitor,[16] were included as positive controls.

Histone Hyperacetylation. MEL cells were incubated with the inhibitors at the concentrations indicated in Figure 2. After 6 h the cells were washed, harvested by centrifugation, and snap-frozen in liquid nitrogen. After thawing, nuclei and subsequently histones were isolated according to standard procedures, and histone hyperacetylation was investigated employing AUT gels as described in the literature.[17,18]

Molecular Modeling. Force-field (MM^+) minimizations of **5g** and **5f** were performed using Hyperchem 5.1 Pro (Hypercube Inc.). Structures were drawn with ISIS-Draw 2.1.4 (MDL Information Systems Inc.) assuming trans-conformations of all amide bonds, imported into Hyperchem, and consecutively minimized (Polak−Ribiere conjugate gradient, in vacuo). A molecular dynamics was performed (300 K, 0.5 ps) followed by another minimization. Then the molecules were superpositioned at the central amide bond.

Acknowledgment. We thank Prof. Unterhalt, Münster, the Fonds der Chemischen Industrie, the Förderergesellschaft der Westfälischen Wilhelms-Universität, the DPhG-Stiftung für den Wissenschaftlichen Nachwuchs im Stifterverband für die Deutsche Wissenschaft, the Deutsche Forschungsgemeinschaft, the Austrian Science Foundation (Grant P-11741), and the Austrian Academy of Sciences for support. We gratefully acknowledge gifts of 6-aminocaproic acid and trichostatin A (Wako) by ASTA Medica AG, Frankfurt. M.J. also thanks H. Deguara for technical assistance.

Note Added in Proof: The crystal structure of a bacterial HD homologue with and without **2** has just recently been published. It proves the zinc dependence of the acetamide cleavage reaction postulated in this paper and its inhibition by hydroxamate chelation (Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* **1999**, *401*, 188−193).

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JM991091H