

# Structure–Activity Relationships on Phenylalanine-Containing Inhibitors of Histone Deacetylase: In Vitro Enzyme Inhibition, Induction of Differentiation, and Inhibition of Proliferation in Friend Leukemic Cells

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Inhibitors of histone deacetylases (HDACs) are a new class of anticancer agents that affect gene regulation. We had previously reported the first simple synthetic HDAC inhibitors with in vitro activity at submicromolar concentrations. Here, we present structure–activity data on modifications of a phenylalanine-containing lead compound including amino acid amides as well as variations of the amino acid part. The compounds were tested for inhibition of maize HD-2, rat liver HDAC, and for the induction of terminal cell differentiation and inhibition of proliferation in Friend leukemic cells. In the amide series, in vitro inhibition was potentiated up to 15-fold, but the potential to induce cell differentiation decreased. Interestingly, an HDAC class selectivity was indicated among some of these amides. In the amino acid methyl ester series, a biphenylalanine derivative was identified as a good enzyme inhibitor, which blocks proliferation in the submicromolar range and is also a potent inducer of terminal cell differentiation.

## Introduction

Histone deacetylase (HDAC) inhibitors are a relatively new class of potential drugs for the treatment of hyperproliferative diseases.<sup>1–3</sup> They induce hyperacetylation of chromatin, which in turn usually leads to the relief of transcriptional repression for a certain subset of genes.<sup>4,5</sup> Among these are genes encoding for proteins that are crucial for the regulation of cell proliferation or differentiation, e.g., the cyclin-dependent kinase inhibitor protein p21/WAF1/CIP1.<sup>6,7</sup> The aberrant recruitment of HDACs by oncogenic fusion proteins seems to be a general mechanism for the pathogenesis of leukemia.<sup>8,9</sup> HDAC inhibitors have demonstrated potential for the prevention and treatment of cancer in numerous cell culture<sup>10</sup> and animal models,<sup>11</sup> and first promising data on humans are available as well.<sup>12</sup> Therefore, HDACs have emerged as an attractive target for new anticancer drugs and there is a great demand for new inhibitors.

Just five years ago, only a few hydrophobic cyclotetrapeptides such as trapoxin B (**1**) and the antifungal antibiotic trichostatin A (**2**) were known as potent inhibitors of HDAC.<sup>13</sup> We had proposed a general model for HDAC inhibitors **3** and have presented the first simple synthetic compounds MD85 (**4a**) and M232 (**5**) that were designed to be potent inhibitors of HDAC according to this model.<sup>14</sup> Our compounds are structurally related to the so-called hybrid polar compounds

such as SAHA (**6a**)<sup>15</sup> and pyroxamide (**6b**).<sup>16</sup> This class of compounds had already been known as inducers of differentiation<sup>17</sup> before the development of our lead compounds and was later identified as HDAC inhibitors. Compounds **6a,b** have now entered phase I clinical trials (Chart 1). We reported on structure–activity data of analogues of **4a** with the most potent compound being its homologue M344 (**4b**), which induced differentiation and inhibited proliferation at 500 nM in Friend leukemic cells.<sup>18</sup> Several interesting compounds from other research laboratories have been added to the group of HDAC inhibitors over the years, but there is still comparatively little structure–activity data available. There are reports on pyrrole analogues of **2**,<sup>19</sup> which are less potent and a series of benzamides with an orally active compound called MS275<sup>20,21</sup> as the most interesting member. Extensive structure–activity data have been reported on analogues of the tetrapeptide apicidin that also displays antiprotozoal activity.<sup>22–27</sup> New reports that aim toward subtype selective HDAC inhibitors have been presented as well.<sup>28,29</sup>

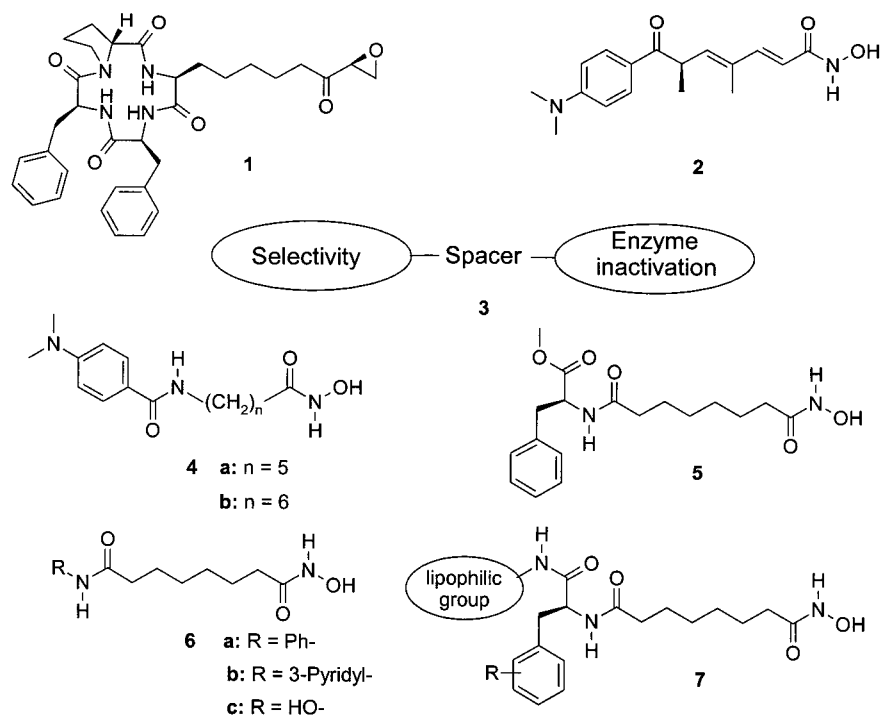
So far, there is only limited structural information on HDACs available. X-ray structures of a bacterial enzyme called HDLP with **2** and **6a** and without an inhibitor have been solved,<sup>30</sup> and two reports on nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent deacetylases, which are not affected by the classical HDAC inhibitors, are available as well.<sup>31,32</sup> HDLP is a homologue of both mammal class enzyme I (homologues of the yeast transcriptional regulator and HDAC rpd3) and mammal class enzyme II (homologues of the yeast transcriptional regulator and HDAC HDA1). The HDLP structure is useful only on the region surrounding the

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**Chart 1.** General Model **3** and Examples of Inhibitors of HDAC (**1**, **2**, and **4–6**); Proposal for Structural Modifications **7**

spacer part of the inhibitors and the catalytic site, which are conserved in mammal class I and II enzymes. Thus, further development is still strongly driven by ligand modification. Here, we report structural modifications of our lead compound **5** and their effect on the inhibition of maize HD-2 and a rat liver HDAC preparation as well as on the inhibition of proliferation and induction of terminal cell differentiation in Friend leukemic cells.

Maize HD-2 had shown a good predictive value in a qualitative and also semiquantitative fashion in the series of amide analogues **4** of trichostatin A (**2**).<sup>18</sup> This had not necessarily to be the case as maize HD-2 is structurally quite different from mammalian HDACs<sup>33</sup> and has been attributed to a HDAC class of its own, which is called class III.<sup>5</sup> The latter term has also been used for the newly discovered class of the  $\text{NAD}^+$ -dependent Sir family.<sup>34</sup> Maize HD-2 is a nucleolar phosphoprotein with sequence homology to other phosphoproteins such as B23 or FKBP, and its active site might be different from the HDLP homologues from classes I and II. Still, it served as a good model in previous studies and we wanted to compare the data from mammal and maize enzymes on a larger series of compounds. The investigations presented here led to an improved inhibitor **11** with a biphenylalanine moiety, which in turn is a promising starting point for further modifications.

### Chemistry

Among our lead compounds **4a** and **5**, the phenylalanine inhibitor **5** was less potent but it displayed an encouraging profile as an inducer of differentiation. We set out to modify this compound as well. As an enzyme-inhibiting group, we continued to use the hydroxamate function. HDAC is a zinc-dependent amidohydrolase, which was only a working hypothesis when we began our first studies. Structure–activity data from our

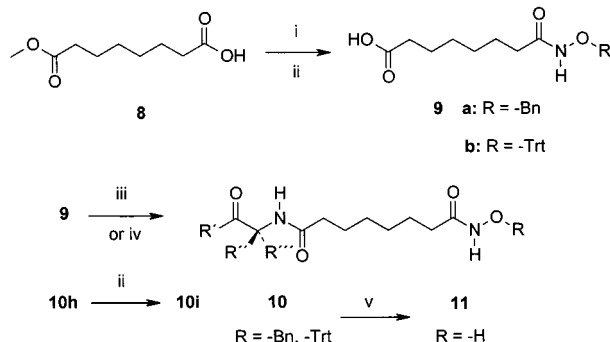
modifications of **4a** supported that hypothesis,<sup>18</sup> but confirmation was only achieved by the X-ray structure of HDLP.<sup>30</sup> Some of the potent inhibitors that were discovered afterward were also hydroxamic acids. The Merck group has shown elegantly in the apicidin series that weak chelators such as carboxylic acids or ketones retain high potency if the rest of the molecule is optimized for binding.<sup>26</sup> A modification of the spacer was not undertaken at this point as it is known from the SAHA series that the suberoyl compounds display the peak of activity.<sup>17</sup> We focused on the region responsible for selective binding to the enzyme, and we have modified the amino acid portion of the molecule as well as the functionality at the “C terminus”. As we postulate a second lipophilic binding site that is occupied by the second phenylalanine in trapoxin B (**1**), we set out to synthesize amide analogues of **5** in order to increase lipophilic contacts with the enzyme as depicted by the general structure **7** (see Chart 1). We also intended to vary the amino acid part of the molecule and to analyze the influence of the stereochemistry on the activity in selected cases.

The synthesis followed the original procedure<sup>14</sup> and in some instances a modified version<sup>35</sup> starting from monomethyl suberate (**8**), which is converted to the protected monohydroxamate **9**. As the target hydroxamic acid **11** is very polar, the synthesis was run in a fashion that the penultimate compound **10** of the synthetic sequence was purified by chromatography and deprotected so that only recrystallization was necessary afterward. Benzyl hydroxylamine and hydrogenation or trityl hydroxylamine and acidic removal were used to generate the desired hydroxamate **11** (see Scheme 1). The amino acids were introduced as the methyl esters and amide hydrochlorides or tosylates. The amino acid amides were synthesized from suitably N-protected amino acids by amide coupling and deprotection. Cbz

**Table 1.** Inhibition of In Vitro Enzyme Activities of Selected HDACs and Proliferation of Cultured Friend Leukemic Cells

no.	R'	R''	R'''	IC <sub>50</sub> ± SD (nM), maize HD-2 (maize HD-1)	IC <sub>50</sub> ± SD (nM), rat liver HDAC	IC <sub>50</sub> (μM), proliferation of Friend leukemic cells
<b>2</b>				3 ± 0.1 <sup>a</sup>	12 ± 1 <sup>b</sup>	0.04 <sup>c</sup>
<b>6a</b>				1000 ± 80 <sup>a</sup>	165 ± 25 <sup>b</sup>	0.99
<b>6c</b>				320 ± 20	1150 ± 55	ND <sup>d</sup>
<b>5</b>	H <sub>3</sub> CO-	Bn-	H-	500 ± 5 <sup>a</sup>	800 ± 130 <sup>e</sup>	15
<b>11a</b>	H <sub>2</sub> N-	Bn-	H-	1100 ± 35	840 ± 95	64
<b>11b</b>	PhNH-	Bn-	H-	80 ± 5 (120 ± 10)	790 ± 70	15
<b>11c</b>	BnNH-	Bn-	H-	140 ± 5 (40 ± 5)	460 ± 30	69
<b>11d</b>	Ph(CH <sub>2</sub> ) <sub>2</sub> NH-	Bn-	H-	330 ± 15 (25 ± 5)	260 ± 20	72
<b>11e</b>	Ph(CH <sub>2</sub> ) <sub>2</sub> NH-	H-	Bn-	340 ± 10	390 ± 25	76
<b>11f</b>	Ph(CH <sub>2</sub> ) <sub>3</sub> NH-	Bn-	H-	380 ± 10	1010 ± 90	58
<b>11g</b>	pyrrolyl-	Bn-	H-	520 ± 35	330 ± 40	> 100
<b>11h</b>	H <sub>3</sub> CO-	H-	Bn-	760 ± 15	880 ± 85	28
<b>11i</b>	HO- <sup>f</sup>	H-	Bn-	750 ± 15	1540 ± 80	> 100
<b>11j</b>	H <sub>3</sub> CO-	4-MeOBn-	H-	250 ± 15	800 ± 90	11
<b>11k</b>	H <sub>3</sub> CO-	4-NO <sub>2</sub> Bn-	H-	850 ± 45	1340 ± 140	35
<b>11l</b>	H <sub>3</sub> CO-	4-PhBn-	H-	210 ± 5	290 ± 25	0.84
<b>11m</b>	H <sub>3</sub> CO-	<i>i</i> Pr-	H-	900 ± 10	380 ± 30	6.2
<b>11n</b>	H <sub>3</sub> CO-	1-naphthyl-methyl-	H-	360 ± 20	130 ± 15	22
<b>11o</b>	H <sub>3</sub> CO-	2-naphthyl-methyl-	H-	40 ± 2	510 ± 40	5.2
<b>11p</b>	H <sub>3</sub> CO-	2-thenyl-	H-	700 ± 35	410 ± 60	5.9
<b>11q</b>	H <sub>3</sub> CO-	3-indolyl-methyl-	H-	120 ± 10	320 ± 20	13
<b>11r</b>	Ph(CH <sub>2</sub> ) <sub>2</sub> NH-	1-naphthyl-methyl-	H-	35 ± 5	310 ± 10	15

<sup>a</sup> Taken from literature.<sup>37</sup> <sup>b</sup> Taken from literature.<sup>52</sup> <sup>c</sup> Taken from literature.<sup>18</sup> <sup>d</sup> Not determined. <sup>e</sup> Taken from literature.<sup>48</sup> <sup>f</sup> As the dicyclohexylamine salt.

**Scheme 1.** Synthetic Route to HDAC Inhibitor **11**<sup>a</sup>

<sup>a</sup> Reagents: (i) BOP-Cl, DIPEA, H<sub>3</sub>NOBn<sup>+</sup>Cl<sup>-</sup>, respectively, H<sub>2</sub>NOTrt, CH<sub>2</sub>Cl<sub>2</sub>, 95% (R = Bn), 78% (R = Trt). (ii) LiOH, H<sub>2</sub>O, THF, and then HCl, 68% (R = Bn), 80% (R = Trt). (iii) *i*BuOCOCl, NMM, H<sub>3</sub>NR<sup>+</sup>X<sup>-</sup> (X = Cl, Tos), THF, 30–86%. (iv) BOP-Cl, DIPEA, H<sub>3</sub>NR<sup>+</sup>X<sup>-</sup> (X = Cl, Tos), CH<sub>2</sub>Cl<sub>2</sub>, 33–69%. (v) H<sub>2</sub>, Pd/C, MeOH for R = Bn, 34–91%; TFA, Et<sub>3</sub>SiH, CH<sub>2</sub>Cl<sub>2</sub> for R = Trt, 42–93%.

or Boc protection were employed, and again, hydrogenation or acidic hydrolysis led to the desired amino acid amide building blocks (see Supporting Information for details). The amino acid methyl esters were prepared from the acids by treatment with thionyl chloride in methanol. To obtain the (*R*)-phenylalanine-derived acid **11i**, the ester function was cleaved with lithium hydroxide in the benzyl-protected precursor **10h** and the resulting acid **11i** was then hydrogenated as with other NHO-benzyl compounds.

**Results and Discussion**

**In Vitro Enzyme Inhibition.** As a source of enzymatic activity, we used a highly purified maize HDAC HD-2<sup>36</sup> and a partially purified rat liver HDAC preparation.<sup>37,38</sup> We have also studied the inhibition of the rpd3 homologue maize HD-1<sup>39</sup> (which is a homologue of mammalian class I HDACs) in selected cases (Table 1). In addition to the large set of comparative data on

human (HeLa) and protozoal (*Eimeria* sp.) enzyme inhibition in the apicidin series, this is the only other report on a larger series of inhibitors with HDACs from different organisms or classes. Standard radiolabeled histones from chicken reticulocytes were used as a substrate for maize enzymes whereas the fluorogenic substrate developed in our group<sup>38</sup> was used with the rat liver deacetylase. Both systems have previously been shown to lead to similar results.<sup>37</sup>

First, we have compared the impact of exchanging the methyl ester substituent in our lead structure **5** by an amide function on the deacetylase inhibitory properties. A Phe-Phe derivative was virtually inactive in both systems (data not shown), which prompted us to test the effect of simple amines on the activity. With regard to maize HD-2, only the primary amide **11a** is significantly less active than the methyl ester **5**. The secondary amides show a steady decrease in enzyme inhibition with an increased number of methylene groups between the phenyl ring and the amide nitrogen. Thus, the anilide **11b** is the most potent phenylalanine amide with an IC<sub>50</sub> of 80 nM. The primary amide **11a** is less active than **5** in the rat system as well. The trend of activity in **11b–f** is altered with the rat liver HDAC preparation as there is an initial activity increase with the number of methylene groups between the phenyl ring and the amide group with a peak at the phenethylamide **11d**. So, we tested the amides **11b–d** also for their inhibition of maize HD-1, which is a homologue of the yeast deacetylase rpd3 and thus mammal class I enzymes. Therefore, it should resemble the rat liver preparation more than maize HD-2 in its inhibitor recognition. The benzylamide **11c** and the phenethylamide **11d** are very similar in their activities, but both are more potent than the anilide **11b**. So, this indicates a reversed structure–activity relationship with respect to maize HD-1 inhibition as compared to maize HD-2 and a similarity to the closer related rat HDAC preparation. The pyrrolidide **11g** as a tertiary amide displays an activity comparable

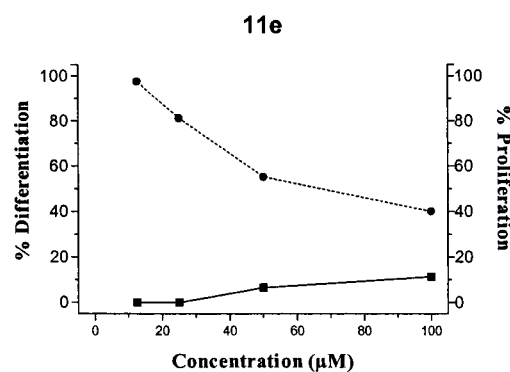
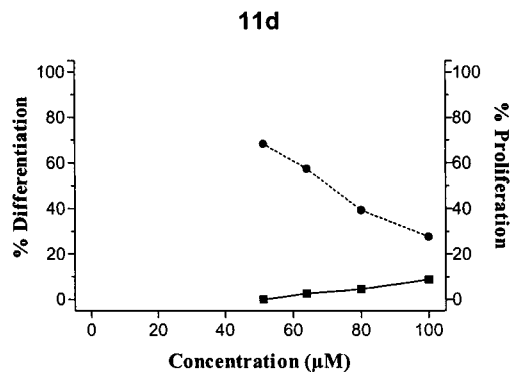
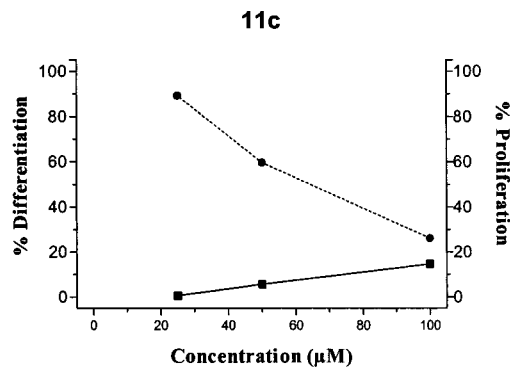
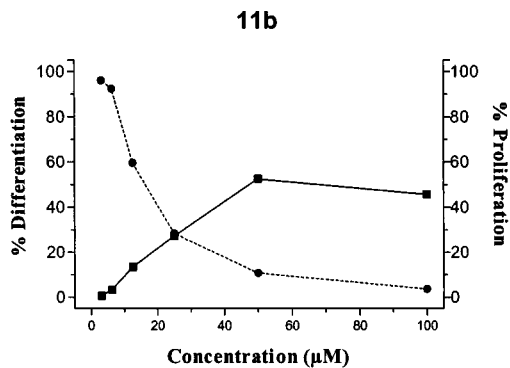
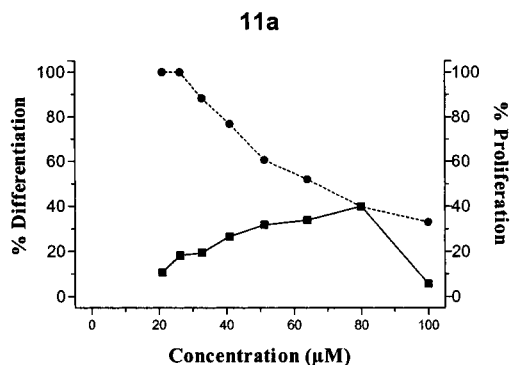
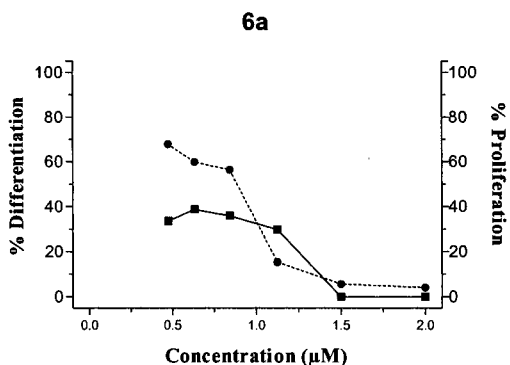
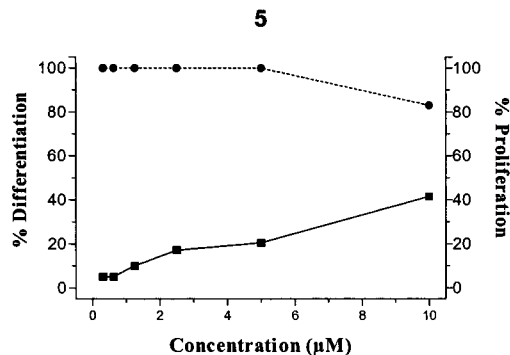
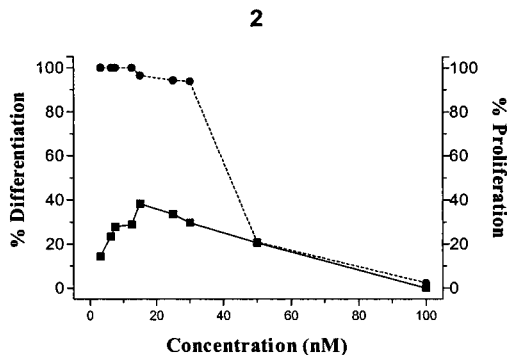
to the methyl ester **5** with maize HD-2, but it is more active in inhibiting rat liver HDAC preparation. Interestingly, the potency of the (*R*)- and (*S*)-phenethylamides (**11d,e**, respectively) is almost identical with maize HD-2 and similar with the rat liver HDAC preparation while both the (*R*)-acid **11i** and -methyl ester **11h** are less active than their enantiomers in both assays (data for (*S*)-acid not shown). Although we have used standard peptide coupling methodology, which should lead only to a minimum of racemization, we nevertheless wanted to rule out that these results stem from complete racemization. We have evaluated several chiral high-performance liquid chromatography (HPLC) stationary phases but could not achieve a complete baseline separation of the two enantiomers. The best separation was monitored on a (*S*)-valine-(*R*)-1-( $\alpha$ -naphthyl)ethylamide phase and led to a limit of quantitation of around 5% of **11e** in **11d** vs 10% of **11d** in **11e**. No sign of the undesired enantiomer was detected in both cases (see Supporting Information for details); therefore, it was proven that the amides **11d,e** display similar HDAC inhibitory properties despite an opposite stereochemistry. As the other compounds in this report are synthesized with similar methods, we do not expect significant racemization in the other inhibitors as well.

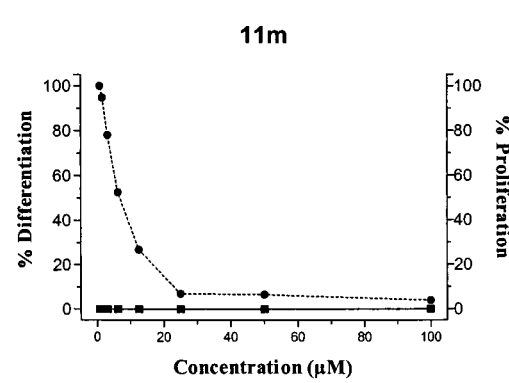
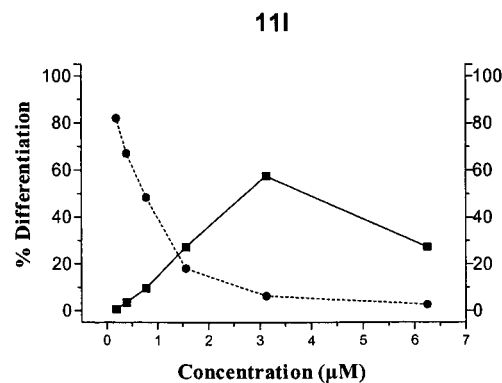
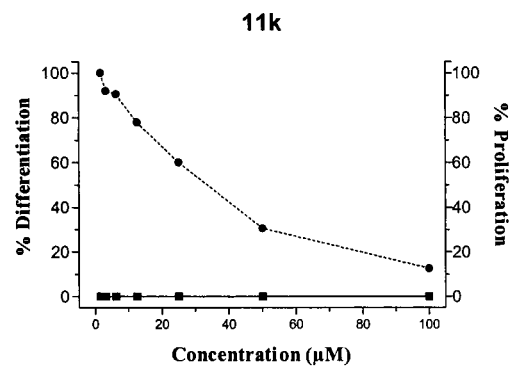
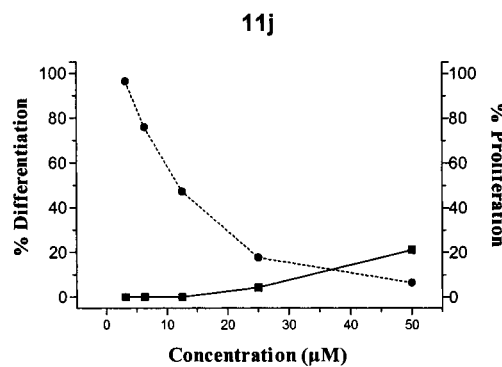
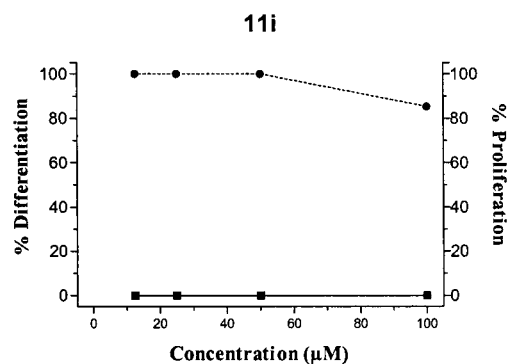
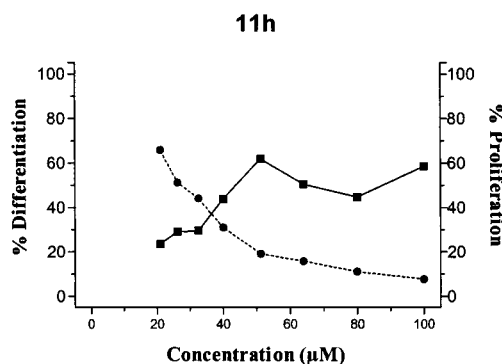
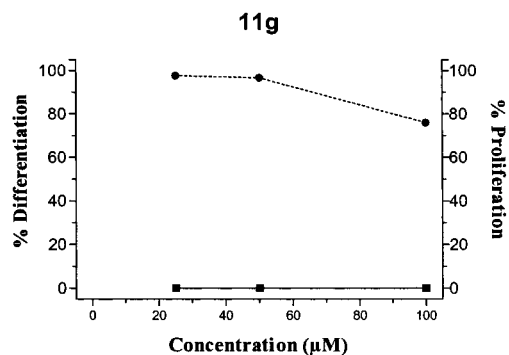
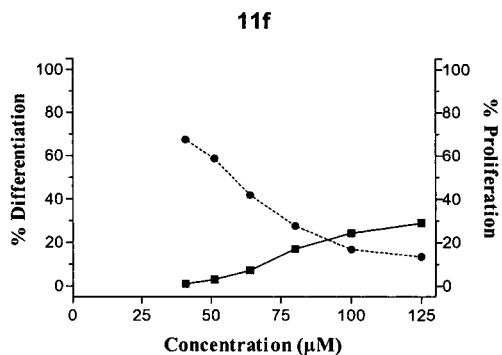
Next, we have modified the amino acid substituent in the phenylalanine methyl ester lead structure **5**. While the methoxyphenylalanine **11j** is more active against HD-2, it is less active in inhibiting the rat liver deacetylase preparation. With the thienyl analogue **11p** and the aliphatic valine derivative **11m**, this order is reversed. The 4-nitrophenylalanine compound **11k** is less active in both systems while the bulky biphenylalanine congener **11l** is about 2-fold more active than the unsubstituted compound in both series. The impact of benzoannellation of the phenyl ring in the naphthyl compounds **11n,o** is dependent on the position of the naphthyl ring. While the hydroxamate derived from 1-naphthylalanine **11n** is more active in both systems and is the best inhibitor in the rat liver deacetylase assay, its regioisomer **11o** is similar in its activity to **5** with rat liver HDAC. Compound **11o** is far more effective on the maize enzyme with an  $IC_{50}$  of 40 nM. Finally, the heteroaromatic tryptophan derivative **11q** is also a good inhibitor of maize HD-2 with an  $IC_{50}$  of 120 nM and it does show an increased rat liver deacetylase inhibition as well. The indole nucleus or suitable replacements in that position have previously been shown to be an important part of potent HDAC inhibitors in the apicidin series.<sup>22</sup> To see whether the combination of the two most successful structural modifications among the amide and the methyl ester series with regard to rat liver HDAC inhibition does lead to a further increase in inhibitory potency, we have synthesized the phenethylamide of 1-naphthylalanine and have converted it to the requisite suberoylamide hydroxamate **11r**. However, it does not reach the activity of either the parent phenethylamide **11d** or the naphthylalanine **11n** in the inhibition of the rat enzyme preparation whereas it is indeed the best inhibitor of maize HD-2 (35 nM). Besides trichostatin A (**2**), the hybrid polar compounds SAHA (**6a**) and SBHA (**6c**) were included as a comparison. We have measured  $IC_{50}$  values of 1000 (maize HD-2) and 165 nM (rat liver

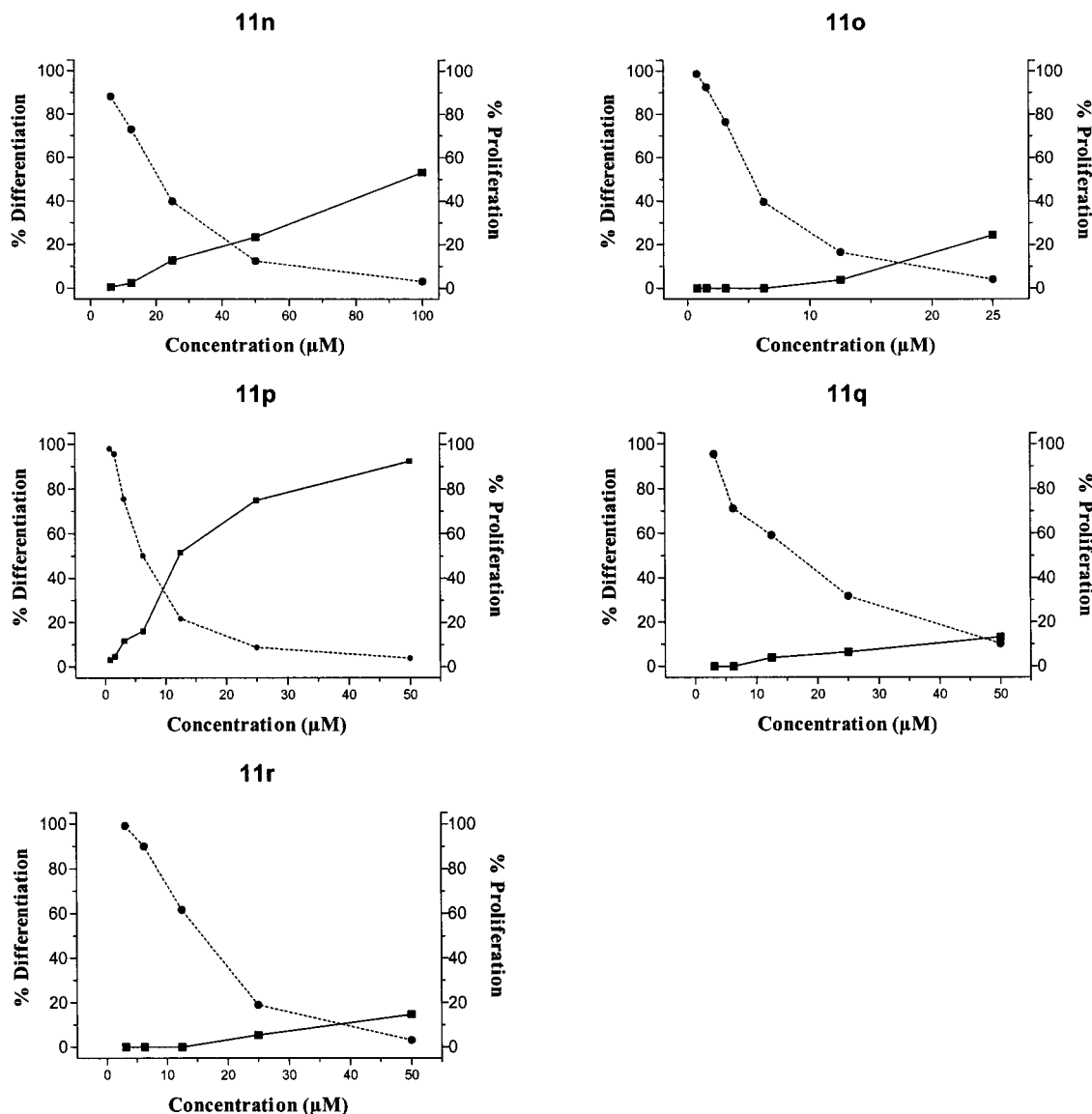
HDAC) for **6a** while for **6c** values of 320 (maize HD-2) and 1150 nM (rat liver HDAC) were obtained. Compound **6a** has been reported to inhibit immunoprecipitated human HDACs 1 and 3 at much lower concentrations (10–20 nM for **6a** and 250–300 nM for **6c**<sup>15</sup>); so, our compounds might display lower values for isolated enzymes as well. On the other hand, the predictive value of those isolated HDAC systems does not seem to be as good as ours for **6a** and the cellular activity is registered in the low micromolar region for both the hybrid polar compounds<sup>17</sup> and our structures (see below).

**Inhibition of Proliferation and Induction of Terminal Cell Differentiation.** For in vivo investigations, Friend leukemic cells were used. They have been found to be a suitable model for induction of differentiation by HDAC inhibitors in many studies.<sup>18,40–42</sup> Friend cell differentiation is accompanied by an accumulation of hemoglobin, which is easily visualized by benzidine staining. Screening tests were performed at 10 and 50  $\mu$ M, and dose–response studies were done according to those initial results. If the compounds were highly toxic at 50  $\mu$ M (>95%), dose–response studies were not extended to that concentration (Figure 1).

By comparing their profiles for inhibition of proliferation and induction of terminal differentiation, the inhibitors could be divided into three classes. The first group consists of the pyrrolidide **11g**, the (*R*)-phenylalanine acid **11i**, the nitrophenylalanine compound **11k**, and the valine derivative **11m**. These compounds do not lead to an induction of differentiation. While **11g,i** are also poor inhibitors of proliferation, **11k,m** do inhibit Friend cell proliferation in a dose-dependent manner. The largest group is characterized by a concentration-dependent inhibition of proliferation and also a dose-dependent induction of terminal differentiation that does not exceed 25% of the surviving cells. These are the inhibitors benzylamide **11c**, phenethylamides **11d,e**, phenylpropylamide **11f**, methoxyalanine **11j**, 2-naphthylalanine **11o**, tryptophan **11q**, and naphthylalanine amide **11r**. The third group is formed by the compounds that lead to a pronounced induction of terminal differentiation in Friend leukemic cells (40% or more of the surviving cells) and are also inhibitors of proliferation. These are the standards trichostatin A (**2**) and SAHA (**6a**), the lead compound **5**, its enantiomer **11h**, the primary amide **11a**, the anilide **11b**, the 1-naphthylalanine methyl ester **11n**, the biphenylalanine compound **11l**, and the thienyl congener **11p**. They show more or less steep dose–response curves with regard to inhibition of proliferation. Generally, a strong induction of differentiation is only detected at concentrations where also marked inhibition of proliferation is encountered. The notable exception to this is the lead compound **5** with 45% induction of differentiation at only 20% inhibition of proliferation (at 10  $\mu$ M) and to some extent its enantiomer **11h**. The cellular activity in the amide series is rather disappointing whereas especially with the more lipophilic and sterically demanding substituents a good induction of differentiation and/or inhibition of proliferation is observed in the amino acid methyl ester series. Maybe a poor uptake of the amides by the cells is a reason for this although their lipophilicity is similar to that of the esters (e.g.,  $\log P = 3.39$  for **11d**, 3.78 for **11f**, 3.01 for **11n**, and 3.69 for **11l**).





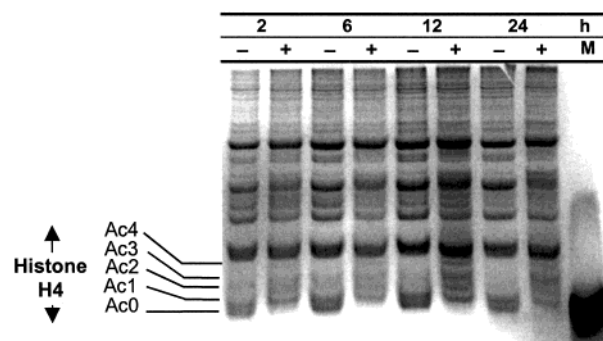


**Figure 1.** Induction of differentiation in Friend leukemic cells by HDAC inhibitors. Circles and dotted lines represent percent proliferation, and squares and straight lines represent percent induction of differentiation in surviving cells. Data for **2** was taken from the literature.<sup>18</sup>

**Histone Hyperacetylation.** We had already demonstrated that the lead compound **5** induces histone hyperacetylation in Friend cells at concentrations where cellular activity is observed as well.<sup>18</sup> To determine whether the mechanism of action is identical with the novel inhibitors, we investigated the influence of the biphenylalanine compound **11l** on the acetylation status of Friend cell histone H4 by employing standard acid-urea-triton (AUT) gels as described in the literature.<sup>43,44</sup> Indeed, a clear time-dependent shift to hyperacetylated species of histone H4 is observed in cells treated with 5 μM **11l**. The peak of hyperacetylation is monitored after 12 h (Figure 2).

### Conclusion

Structural modifications of our phenylalanine methyl ester lead compound **5** have resulted in a potentiation of the enzyme inhibitory properties in both maize HD-2 and rat liver HDAC preparation. While the inhibition of maize HD-2 was especially pronounced in the amino acid amide series, resulting in an up to 15-fold increase



**Figure 2.** Time-dependent acetylation status of histone H4 in Friend leukemic cells treated with 5 μM of **11l**. A + represents treated cells, and a - represents solvent control. Ac0–4, number of acetylated lysines; M, marker (Cytochrome c). See the Experimental Section for details.

in inhibitory potential, an increase of the size of the amino acid substituent led to the best results in the rat liver system. The rise in activity in this assay was limited to a factor of three. The results from the

phenylalanine amides **11b–d** (with the additional data on maize HD-1) indicate a HDAC subtype or class selectivity, but certainly tests with pure mammalian HDAC subtypes are necessary to substantiate this. Both models were able to identify inhibitors of proliferation and inducers of differentiation in leukemic cells in a primary in vitro screen. Thus, the importance of HDAC assays as a tool for drug development is further emphasized. The semiquantitative prediction of cellular activity was not as consistent in this series as compared to the amide analogues of trichostatin A (**2**).<sup>18</sup> The amides are mostly moderately active in the Friend leukemic cells, but better predictive results are obtained in the ester series with the good inhibitors **11l,n** being also among the most active compounds in cell culture. While the difference in cellular activity cannot be attributed to a simple difference in lipophilicity, still a differential uptake of these compounds might be a reason for the observed results. Additionally, a different selectivity in HDAC subtype inhibition might account for the varying cellular activity profiles in seemingly very similar compounds. Such alternative binding modes even for simple homologues have been shown on a homology model of human HDAC1 that is based on the HDLP X-ray structure.<sup>45</sup> So, the observed IC<sub>50</sub> in the rat liver system might be a result of a differential inhibition of various HDAC subtypes. Still, the rat liver HDAC is a convenient and accessible screening tool and the example of **6a** shows that highly purified HDACs are not necessarily better in quantitative predictions of cellular activity. Another explanation for the observed discrepancies might be the involvement of additional mechanisms apart from histone hyperacetylation by different hydroxamic acids in other pathways. The hydroxamic acid moiety is able to chelate iron and zinc ions and might interact with other enzymes, but at least for the lead compound **5**, it was shown specifically that it does not inhibit a number of matrix metalloproteases in concentrations where histone hyperacetylation occurs (unpublished results). Generally, the different profiles of cellular activity in structurally similar compounds (inhibition of proliferation vs induction of differentiation) should stimulate further work.

**Summary.** The best compound from a series of structural modifications of a lead HDAC inhibitor with a phenylalanine substructure was the biphenylalanine methyl ester derivative **11l** with an IC<sub>50</sub> below 1  $\mu$ M causing inhibition of proliferation and induction of terminal cell differentiation in Friend leukemic cells. This compound is also inducing histone hyperacetylation at low micromolar concentrations and opens up new routes for further systematic improvement, e.g., by palladium-mediated biaryl coupling.

## Experimental Section

Melting points are uncorrected. Elemental analysis was performed on a Foss-Heraeus CHN-O-Rapid. IR spectra were recorded on a Shimadzu 470 in KBr or a Biorad FTS 135 in KBr. <sup>1</sup>H nuclear magnetic resonance (NMR) was done on a Varian Gemini 200 (200 MHz) and <sup>13</sup>C NMR on the same instrument (50.29 MHz). MS spectrometry was done on a Finnigan MAT 312 (EI). Flash chromatography was performed using silica gel 60, 230–400 mesh (Merck). Dichloromethane and tetrahydrofuran (THF) were dried over molecular sieves (3 Å). Shimadzu RF 535 was used as fluorescence detector for HPLC and a LiChrosorb RP 18 5  $\mu$ m (125 mm  $\times$  3 mm,

Knauer) column was used in the assay. Trichostatin A (**2**) and SAHA (**6a**) were purchased from Calbiochem, and SBHA (**6c**) was from Aldrich. Phenylalanine pyrrolidide was purchased from Bachem. Amino acids were obtained from Calbiochem, and other chemicals were from Fluka and Aldrich. Compounds **5** and **9a** were synthesized according to the literature.<sup>35</sup> Dulbecco's modified Eagle's medium (DMEM), penicillin G sodium, and streptomycin sulfate were purchased from Life Technologies. Log $P$  values were calculated using HyperChem Pro 6.02.

**Synthesis of Inhibitors. Amino Acid Building Blocks.** The amino acid methyl esters were prepared from the commercially available acids using methanol/thionyl chloride according to standard procedures. The amino acid amide salts were prepared from the amines and commercially available Boc- or Cbz-protected amino acids via mixed anhydride coupling and subsequent hydrogenation (Cbz) or acidic removal of the protecting group (trifluoroacetic acid, Boc). The liberated bases were treated with HCl in ether or *p*-toluenesulfonic acid in ethyl acetate to precipitate the salts. See Supporting Information for details and spectroscopic data.

**Method A. Amide Formation Using BOP-Cl.** To a suspension or solution of the acid in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL/mmol) was added diisopropyl ethylamine (DIPEA, 1 equiv) under nitrogen, and the mixture was stirred for 10 min. Then, BOP-Cl (1 equiv), the  $\alpha$ -amino acid methyl ester hydrochloride (or appropriately protected hydroxylamine) (1.1 equiv) and again DIPEA (2 equiv) were added. After it was stirred overnight, most of the CH<sub>2</sub>Cl<sub>2</sub> was removed under reduced pressure and ethyl acetate (100 mL) was added. The solution was washed three times with 5% NaHCO<sub>3</sub> solution and once consecutively with water, 2 M HCl solution, water and saturated brine (50 mL each). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent was evaporated.

**Method B. Amide Formation Using Mixed Anhydride.** To a solution of the acid in THF (10 mL/mmol) was added *N*-methyl morpholine (NMM, 1 equiv) under nitrogen, and the solution was stirred for 5 min. The solution was cooled to -15  $^{\circ}$ C and stirred for another 5 min. Then, isobutyl chloroformate (1 equiv) was added dropwise, and the mixture was stirred for 10 min. The amine (or the  $\alpha$ -amino acid methyl ester hydrochloride) (1 equiv) and again NMM (2 equiv) were added, and the suspension was stirred for 15 min at -15  $^{\circ}$ C and 2 h at room temperature. The mixture was then poured in 50 mL of 2 M HCl solution and extracted three times with ethyl acetate (each 50 mL). The organic layers were then washed with 5% NaHCO<sub>3</sub> solution, water, and saturated brine (each 50 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, most of the solvent was evaporated, and the crude product was precipitated with hexane.

**Method C. Ester Cleavage.** The ester was dissolved in THF (2 mL/mmol), and an aqueous solution of LiOH (0.5 M, 2 equiv) was added. The solution was stirred overnight at room temperature. To the mixture was then added water (20 mL) and ethyl acetate (50 mL). The aqueous layer was acidified with 6 M HCl dropwise (to pH 3) and consecutively extracted with ethyl acetate (3  $\times$  50 mL). The organic layer was washed with water and saturated brine and dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent was evaporated.

**Method D. Hydrogenation.** The *N*-benzyl precursor was dissolved in methanol (10 mL/mmol), and 10% palladium on charcoal (10%, w/w) was added. The mixture was treated with hydrogen under atmospheric pressure for 4 h and was filtered subsequently. Then, the solvent was evaporated. The hydroxamic acids were redissolved in methanol and precipitated with diethyl ether.

**Method E. Removal of Trt Protecting Groups.** The trityl precursor was dissolved in CH<sub>2</sub>Cl<sub>2</sub>, and trifluoroacetic acid/CH<sub>2</sub>Cl<sub>2</sub> (1:1, v/v; 2 mL/mg) was added. The resulting yellow solution was treated dropwise with triethylsilane until the color disappeared. The mixture was then stirred for 2 h, and the solvent was evaporated afterward. The residue was redissolved with methanol, and the solvent was removed under reduced pressure again. This process was repeated three times,



and the product was finally precipitated with diethyl ether from the solution in methanol.

**7-Trityloxycarbamoyl-heptanoic Acid Methyl Ester.** This was synthesized by method A from monomethyl suberate (**8**) (2.5 g, 13.3 mmol), DIPEA (2.30 mL, 1.72 g, 13.3 mmol), O-tritylhydroxylamine (4.0 g, 14.6 mmol), and DIPEA (4.60 mL, 3.44 g, 26.6 mmol). The crude product was chromatographed using ethyl acetate/hexane (2:1); yield 4.6 g (78%); mp 96 °C. IR: 1740, 1665. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.34–7.26 (m, 15H), 3.66 (s, 3H), 2.26 (t, *J* = 7.5 Hz, 2H), 1.62–1.47 (m, 4H), 1.39–0.85 (m, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 174.15, 141.50, 129.13, 128.13, 128.03, 51.42, 34.06, 31.37, 28.79, 28.75, 24.77, 23.46. MS (EI): *m/z* 243 (Trt<sup>+</sup>).

**7-Trityloxycarbamoyl-heptanoic Acid (9b).** Compound **9b** was synthesized by method C from 7-trityloxycarbamoyl-heptanoic acid methyl ester (3.9 g, 8.7 mmol); yield 3.0 g (80%); mp 150 °C. IR: 1739, 1679. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.34–7.21 (m, 15H), 2.27 (t, *J* = 7.5 Hz, 2H), 1.57–1.46 (m, 4H), 1.30–1.07 (m, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 178.57, 141.45, 129.14, 128.16, 109.49, 33.85, 28.70, 24.53. MS (EI): *m/z* 243 (Trt<sup>+</sup>), 165 (C<sub>13</sub>H<sub>9</sub><sup>+</sup>).

**(S)-Octanedioic Acid Benzyloxy-amide (1-Carbamoyl-2-phenyl-ethyl)amide (10a).** Compound **10a** was synthesized by method A from **9a** (300 mg, 1.07 mmol), DIPEA (0.185 mL, 138.3 mg, 1.07 mmol), BOP-Cl (272 mg, 1.07 mmol), L-phenylalanine amide hydrochloride (236 mg, 1.18 mmol), and DIPEA (0.555 mL, 415 mg, 3.21 mmol); yield 148 mg (33%); mp 173 °C. IR: 1674, 1637, 1543. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.93 (s, 1H), 7.92 (d, *J* = 8.49 Hz, 1H), 7.42–7.12 (m, 10H), 7.03 (s, 2H), 4.76 (s, 2H), 4.47–4.40 (m, 1H), 3.03–2.94 (m, 1H), 2.77–2.65 (m, 1H), 2.03–1.86 (m, 4H), 1.40–1.25 (m, 4H), 1.07–0.99 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 173.34, 171.93, 169.37, 136.08, 138.18, 129.09, 128.72, 128.25, 128.17, 127.92, 126.10, 76.72, 53.58, 37.62, 35.18, 32.21, 28.25, 28.15, 25.05, 24.79. MS (EI): *m/z* 425 (M<sup>+</sup>).

**(S)-Octanedioic Acid (1-Carbamoyl-2-phenyl-ethyl)-amide Hydroxyamide (11a).** Compound **11a** was synthesized by method D from **10a** (150 mg, 0.352 mmol) and 10% Pd on charcoal (15 mg); yield 40 mg (34%); mp 138 °C. IR: 1675, 1630. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.33 (s, 1H), 8.66 (s, 1H), 7.92 (d, *J* = 8.38 Hz, 1H), 7.22–7.15 (m, 5H), 7.03 (s, 2H), 4.43–4.38 (m, 1H), 3.02–2.93 (m, 1H), 2.76–2.68 (m, 1H), 2.03–1.85 (m, 4H), 1.43–1.29 (m, 4H), 1.11–1.04 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 173.23, 171.85, 169.05, 138.11, 128.99, 127.85, 126.03, 53.51, 37.54, 35.11, 32.17, 28.29, 28.11, 24.96. MS (EI): *m/z* 120 (C<sub>8</sub>H<sub>10</sub>N<sup>+</sup>). Anal. (C<sub>17</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub>) C, H, N.

**(S)-Octanedioic Acid Benzyloxyamide (2-Phenyl-1-phenylcarbamoyl-ethyl)amide (10b).** Compound **10b** was synthesized by method B from **9a** (300 mg, 1.07 mmol), NMM (0.117 mL, 108 mg, 1.07 mmol), isobutyl chloroformate (0.140 mL, 137 mg, 1.07 mmol), (*S*)-2-amino-3-*N*-diphenyl-propionamide tosylate (441 mg, 1.07 mmol), and NMM (0.234 mL, 216 mg, 2.14 mmol); yield 160 mg (30%); mp 165 °C. IR: 1655, 1641, 1536. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 7.48–7.08 (m, 15H), 4.82 (s, 2H), 4.82–4.70 (m, 1H), 3.21–3.11 (m, 1H), 3.01–2.90 (m, 1H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.00 (t, *J* = 7.1 Hz, 2H), 1.51–1.48 (m, 4H), 1.28–1.17 (m, 4H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 176.11, 138.29, 130.34, 129.76, 129.65, 129.45, 127.83, 125.50, 121.59, 78.92, 56.68, 39.21, 36.66, 33.66, 29.58, 26.64, 26.40. MS (EI): 120 (C<sub>8</sub>H<sub>10</sub>O<sup>+</sup>), 105 (C<sub>8</sub>H<sub>9</sub><sup>+</sup>).

**(S)-Octanedioic Acid Hydroxyamide (2-Phenyl-1-phenylcarbamoyl-ethyl)amide (11b).** Compound **11b** was synthesized by method D from **10b** (100 mg, 0.20 mmol) and 10% Pd on charcoal (10 mg); yield 50 mg (61%); mp 141 °C. IR: 1642, 1534. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.14 (s, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 7.61–7.05 (m, 10H), 4.78–4.55 (m, 1H), 3.09–2.84 (m, 2H), 2.09–1.88 (m, 4H), 1.45–1.26 (m, 4H), 1.14–1.06 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.17, 170.30, 169.09, 138.78, 137.66, 129.08, 128.58, 127.92, 126.20, 123.29, 119.34, 54.64, 37.66, 35.04, 32.18, 28.29, 28.13, 25.01, 24.92. MS (EI): *m/z* 120 (C<sub>8</sub>H<sub>10</sub>N<sup>+</sup>). Anal. (C<sub>23</sub>H<sub>29</sub>N<sub>3</sub>O<sub>4</sub>) C, H, N.

**(S)-Octanedioic Acid (1-Benzylcarbamoyl-2-phenyl-ethyl)amide Trityloxy-amide (10c).** Compound **10c** was synthesized by method B from **9b** (450 mg, 1.04 mmol), NMM

(0.114 mL, 105 mg, 1.04 mmol), isobutyl chloroformate (0.136 mL, 142 mg, 1.04 mmol), (*S*)-2-amino-*N*-benzyl-3-phenyl-propionamide hydrochloride (302 mg, 1.04 mmol), and NMM (0.228 mL, 210 mg, 2.08 mmol); yield 410 mg (59%); mp 156 °C. IR: 1656, 1544. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.80–7.03 (m, 25H), 4.67 (m, 1H), 4.41–4.19 (m, 2H), 3.15–3.03 (m, 2H), 2.11–2.04 (m, 2H), 1.57–1.38 (m, 4H), 1.25–1.08 (m, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 173.09, 170.89, 137.68, 136.81, 129.36, 129.14, 128.74, 128.69, 128.14, 127.71, 127.52, 127.03, 54.72, 43.60, 38.61, 36.39, 28.68, 25.29, 23.20. MS (EI): *m/z* 243 (Trt<sup>+</sup>).

**(S)-Octanedioic Acid (1-Benzylcarbamoyl-2-phenyl-ethyl)amide Hydroxyamide (11c).** Compound **11c** was synthesized by method E from **10c** (200 mg, 0.299 mmol); yield 85 mg (67%); mp 168 °C. IR: 1638, 1546. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.32 (s, 1H), 8.66 (s, 1H), 8.46 (t, *J* = 5.8 Hz, 1H), 8.05 (d, *J* = 8.3 Hz, 1H), 7.28–7.12 (m, 10H), 4.55–4.53 (m, 1H), 4.25 (d, *J* = 5.8 Hz, 2H), 3.04–2.95 (m, 1H), 2.82–2.70 (m, 1H), 2.06–1.86 (m, 4H), 1.40–1.34 (m, 4H), 1.10 (bs, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 171.94, 171.19, 169.05, 139.11, 137.89, 129.03, 128.08, 127.88, 126.96, 126.56, 126.08, 53.88, 41.96, 37.68, 35.11, 32.19, 28.29, 28.13, 24.98, 24.89. MS (EI): *m/z* 425 (M<sup>+</sup>). Anal. (C<sub>24</sub>H<sub>31</sub>N<sub>3</sub>O<sub>4</sub>) C, H, N.

**(S)-Octanedioic Acid Benzyloxy-amide (1-Phenethyl-carbamoyl-2-phenyl-ethyl)amide (10d).** Compound **10d** was synthesized by method B from **9a** (300 mg, 1.07 mmol), NMM (0.117 mL, 108 mg, 1.07 mmol), isobutyl chloroformate (0.140 mL, 137 mg, 1.07 mmol), (*S*)-2-amino-*N*-phenethyl-3-phenyl-propionamide tosylate (471 mg, 1.07 mmol), and NMM (0.234 mL, 216 mg, 2.14 mmol); yield 420 mg (74%); mp 160 °C. IR: 1638, 1545. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 7.39–7.13 (m, 15H), 4.83 (s, 2H), 4.55 (dd, *J* = 9.10/6.10 Hz, 1H), 3.41–3.26 (m, 2H), 3.09–2.99 (m, 1H), 2.86–2.74 (m, 1H), 2.69 (t, *J* = 7.45 Hz, 2H), 2.12 (t, *J* = 7.30 Hz, 2H), 2.00 (t, *J* = 7.30 Hz, 2H), 1.54–1.37 (m, 4H), 1.28–1.16 (m, 4H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 175.85, 173.56, 140.37, 138.55, 130.27, 129.80, 129.61, 129.49, 129.43, 127.76, 78.95, 56.02, 41.94, 39.14, 36.14, 36.75, 36.43, 33.70, 29.66, 29.56, 26.36. MS (EI): *m/z* 529 (M<sup>+</sup>).

**(S)-Octanedioic Acid Hydroxyamide (1-Phenethylcarbamoyl-2-phenyl-ethyl)amide (11d).** Compound **11d** was synthesized by method D from **10d** (360 mg, 0.680 mmol) and 10% Pd on charcoal (36 mg); yield 180 mg (60%); mp 119 °C. IR: 1637, 1542. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.33 (s, 1H), 8.66 (s, 1H), 8.05–7.95 (m, 2H), 7.30–7.15 (m, 10H), 4.47–4.38 (m, 1H), 3.36–3.19 (m, 2H), 2.93–2.84 (m, 1H), 2.73–2.61 (m, 3H), 2.03–1.86 (m, 4H), 1.40–1.29 (m, 4H), 1.11–1.04 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 171.85, 171.07, 169.06, 139.28, 137.97, 128.99, 128.52, 128.18, 127.85, 126.05, 125.96, 53.79, 40.05, 37.72, 35.10, 34.95, 32.19, 28.27, 28.11, 24.96, 24.89. MS (EI): *m/z* 439 (M<sup>+</sup>). Anal. (C<sub>25</sub>H<sub>33</sub>N<sub>3</sub>O<sub>4</sub>) C, H, N.

**(R)-Octanedioic Acid Benzyloxy-amide (1-Phenethyl-carbamoyl-2-phenyl-ethyl)amide (10e).** Compound **10e** was synthesized by method B from **9a** (300 mg, 1.07 mmol), NMM (0.117 mL, 108 mg, 1.07 mmol), isobutyl chloroformate (0.140 mL, 146 mg, 1.07 mmol), (*R*)-2-amino-*N*-phenethyl-3-phenyl-propionamide tosylate (471 mg, 1.07 mmol), and NMM (0.234 mL, 216 mg, 2.14 mmol); yield 340 mg (60%); mp 164 °C. IR 1638, 1546. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 7.41–7.13 (m, 15H), 4.83 (s, 2H), 4.55 (dd, *J* = 9.15/6.10 Hz, 1H), 3.40–3.26 (m, 2H), 3.09–2.99 (m, 1H), 2.86–2.74 (m, 1H), 2.69 (t, *J* = 7.45 Hz, 2H), 2.12 (t, *J* = 7.38 Hz, 2H), 2.00 (t, *J* = 7.25 Hz, 2H), 1.54–1.37 (m, 4H), 1.18–1.10 (m, 4H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 175.83, 173.56, 140.35, 138.53, 130.25, 129.78, 129.60, 129.47, 129.41, 127.74, 127.34, 78.93, 56.01, 41.92, 39.12, 36.74, 36.41, 33.66, 29.60, 26.55, 26.34. MS (EI): *m/z* 529 (M<sup>+</sup>).

**(R)-Octanedioic Acid Hydroxyamide (1-Phenethylcarbamoyl-2-phenyl-ethyl)amide (11e).** Compound **11e** was synthesized by method D from **10e** (300 mg, 0.566 mmol) and 10% Pd on charcoal (30 mg); yield 190 mg (76%); mp 126 °C. IR: 1640, 1549. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.32 (s, 1H), 8.66 (s, 1H), 8.04–7.95 (m, 2H), 7.30–7.15 (m, 10H), 4.46–4.39 (m, 1H), 3.36–3.19 (m, 2H), 2.93–2.84 (m, 1H), 2.73–2.61 (m, 3H), 2.03–1.85 (m, 4H), 1.43–1.25 (m, 4H), 1.09 (bs, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 171.85, 171.07, 169.06, 139.28, 137.97, 128.99, 128.52, 128.18, 127.85, 126.05, 125.96, 53.79, 40.05, 37.72,

35.10, 34.95, 32.19, 28.27, 28.11, 24.96, 24.89. MS (EI):  $m/z$  439 ( $M^+$ ). Anal. ( $C_{25}H_{33}N_3O_4$ ) C, H, N.

**(S)-Octanedioic Acid Benzyloxyamide [2-Phenyl-1-(3-phenyl-propylcarbamoyl)ethyl]amide (10f).** Compound **10f** was synthesized by method A from **9a** (300 mg, 1.07 mmol), DIPEA (0.185 mL, 138 mg, 1.07 mL), BOP-Cl (272 mg, 1.07 mmol), (S)-2-amino-3-phenyl-*N*-(3-phenyl-propyl)propionamide tosylate (536 mg, 1.18 mmol), and DIPEA (0.555 mL, 414 mg, 3.21 mmol); yield 300 mg (52%); mp 159 °C.  $^1H$  NMR ( $CD_3OD$ ):  $\delta$  7.39–7.11 (m, 15H), 4.60 (s, 2H), 4.60–4.53 (m, 1H), 3.15–3.02 (m, 3H), 2.92–2.80 (m, 1H), 2.54–2.47 (m, 2H), 2.18–2.11 (m, 2H), 2.03–1.96 (m, 2H), 1.73–1.65 (m, 2H), 1.60–1.33 (m, 4H), 1.34–1.02 (m, 4H).  $^{13}C$  NMR ( $CD_3OD$ ):  $\delta$  175.95, 173.54, 172.89, 142.95, 138.48, 136.98, 130.29, 129.61, 129.43, 129.34, 127.78, 126.85, 78.93, 56.15, 39.97, 39.14, 36.72, 34.01, 33.66, 32.04, 29.60, 26.62, 26.36. MS (EI):  $m/z$  543 ( $M^+$ ).

**(S)-Octanedioic Acid Hydroxyamide [2-Phenyl-1-(3-phenyl-propylcarbamoyl)ethyl]amide (11f).** Compound **11f** was synthesized by method D from **10f** (250 mg, 0.450 mmol) and 10% Pd on charcoal (25 mg); yield 190 mg (91%); mp 115 °C. IR: 1761, 1635, 1545.  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  10.36 (s, 1H), 8.68 (s, 1H), 8.05–7.99 (m, 2H), 7.30–7.15 (m, 10H), 4.48–4.45 (m, 1H), 3.04–2.68 (m, 4H), 2.53–2.45 (m, 2H), 2.05–1.86 (m, 4H), 1.70–1.59 (m, 2H), 1.40–1.31 (m, 4H), 1.10–1.09 (m, 4H).  $^{13}C$  NMR (DMSO- $d_6$ ):  $\delta$  171.90, 171.07, 169.10, 141.66, 137.95, 129.03, 128.19, 128.14, 127.87, 126.06, 125.59, 56.02, 38.04; 37.81, 35.13, 32.29, 32.19, 30.67, 28.29, 28.15, 25.02, 24.91. MS (EI):  $m/z$  453 ( $M^+$ ). Anal. ( $C_{26}H_{35}N_3O_4$ ) C, H, N.

**(S)-Octanedioic Acid (1-Benzyl-2-oxo-2-pyrrolidin-1-ethyl)amide Trityloxyamide (10g).** Compound **10g** was synthesized by method B from **9b** (500 mg, 1.16 mmol), NMM (0.128 mL, 117 mg, 1.16 mmol), isobutyl chloroformate (0.151 mL, 158 mg, 1.16 mmol), *l*-phenylalanine-pyrrolidide (253 mg, 1.16 mmol), and NMM (0.255 mL, 234 mg, 2.32 mmol). The crude product was chromatographed using ethyl acetate/methanol (25:1); yield 320 mg (44%); mp 90 °C. IR: 1630, 1449.  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  7.77 (bs, 1H), 7.17–7.45 (m, 20H), 6.40 (d,  $J = 8.2$  Hz, 1H), 4.84–4.96 (m, 1H), 3.29–3.38 (m, 3H), 2.93–3.01 (m, 2H), 2.54–2.59 (m, 1H), 2.09–2.16 (m, 2H), 0.83–1.71 (m, 14H).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  172.40, 169.78, 136.46, 129.51, 129.13, 128.43, 127.03, 52.24, 46.40, 45.82, 39.81, 36.50, 28.84, 24.07, 25.82, 25.35. MS (EI):  $m/z$  243 ( $Trt^+$ ).

**(S)-Octanedioic Acid (1-Benzyl-2-oxo-2-pyrrolidin-1-ethyl)amide Hydroxyamide (11g).** Compound **11g** was synthesized by method E from **10g** (300 mg, 0.48 mmol); yield 110 mg (59%) viscous oil. IR: 1625, 1455.  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  7.42–7.38 (m, 5H), 5.09–4.85 (m, 1H), 3.69–3.47 (m, 3H), 3.20–3.18 (m, 2H), 2.87–2.78 (m, 1H), 2.42–2–31 (m, 3H), 1.93–1.75 (m, 6H), 1.45 (bs, 3H).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  173.84, 171.03, 136.74, 129.72, 128.79, 127.37, 53.09, 46.88, 46.35, 39.11, 35.86, 32.53, 28.02, 26.08, 25.10, 25.40, 24.34. MS (EI):  $m/z$  120 ( $C_8H_{10}O^+$ ), 201 ( $C_{13}H_{15}NO^+$ ). Anal. ( $C_{21}H_{31}N_3O_4 \cdot 0.5H_2O$ ) C, H, N.

**(R)-2-(7-Benzyloxycarbamoyl-heptanoylamino)-3-phenyl-propionic Acid Methyl Ester (10h).** Compound **10h** was synthesized by method A from **9a** (670 mg, 2.4 mmol), DIPEA (0.415 mL, 310 mg, 2.40 mmol), BOP-Cl (611 mg, 2.40 mmol), *D*-phenylalanine methyl ester hydrochloride (569 mg, 2.64 mmol), and DIPEA (1.25 mL, 931 mg, 7.20 mmol); yield 730 mg (69%); mp 89 °C. IR: 1733, 1641, 1521.  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  8.28 (bs, 1H), 7.38–7.06 (m, 10H), 5.87 (d,  $J = 7.64$  Hz, 1H), 4.91–4.83 (m, 3H), 3.72 (s, 3H), 3.14–3.04 (m, 2H), 2.18–2.00 (m, 4H), 1.62–1.50 (m, 4H), 1.29–1.26 (m, 4H).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  172.64, 172.5, 135.97, 129.30, 128.87, 128.68, 127.24, 53.04, 52.40, 37.99, 36.27, 28.44, 25.22. MS (EI):  $m/z$  162 ( $C_{10}H_{10}O_2^+$ ).

**(R)-2-(7-Hydroxycarbamoyl-heptanoylamino)-3-phenyl-propionic Acid Methyl Ester (11h).** Compound **11h** was synthesized by method D from **10h** (200 mg, 0.45 mmol) and 10% Pd on charcoal (20 mg); yield 110 mg (70%); mp 109 °C. IR: 1739, 1662, 1626.  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  10.32 (s, 1H), 8.66

(s, 1H), 8.25 (d,  $J = 7.92$  Hz, 1H), 7.26–7.18 (m, 5H), 4.46–4.43 (m, 1H), 3.58 (s, 3H), 2.99–2.85 (m, 2H), 1.86–2.05 (m, 4H), 1.39–1.36 (m, 4H), 1.13–1.12 (m, 4H).  $^{13}C$  NMR (DMSO- $d_6$ ):  $\delta$  172.19, 169.06, 137.27, 128.95, 128.11, 126.41, 53.31, 51.70, 34.89, 32.21, 28.23, 28.11, 24.97. MS (EI):  $m/z$  350 ( $M^+$ ). Anal. ( $C_{18}H_{26}N_2O_5$ ) C, H, N.

**(R)-2-(7-Benzyloxycarbamoyl-heptanoylamino)-3-phenyl-propionic Acid (10i).** Compound **10i** was synthesized by method C from **10h** (510 mg, 19.5 mmol); yield 410 mg (83%); mp 115 °C. IR: 1742, 1641, 1541.  $^1H$  NMR ( $CD_3OD$ ):  $\delta$  7.44–7.18 (m, 10H), 4.83 (s, 2H), 4.70–4.63 (m, 1H), 3.27–3.17 (m, 1H), 2.97–2.85 (m, 1H), 2.16–1.97 (m, 4H), 1.49–1.43 (m, 4H), 1.24–1.12 (m, 4H).  $^{13}C$  NMR ( $CD_3OD$ ):  $\delta$  176.01, 174.85, 138.59, 130.32, 130.23, 129.69, 129.42, 127.78, 78.94, 54.86, 38.4, 36.66, 33.70, 29.69, 29.59, 26.63, 26.42. MS (EI):  $m/z$  426 ( $M^+$ ).

**(R)-2-(7-Hydroxycarbamoyl-heptanoylamino)-3-phenyl-propionic Acid (11i).** Compound **11i** was synthesized by method D from **10i** (200 mg, 0.45 mmol) and 10% Pd on charcoal (20 mg); yield 65 mg (41%) of viscous oil. The acid was precipitated from a solution in methanol with an excess of dicyclohexylamine (dropwise addition of a solution in diethyl ether). IR: 2937, 2858, 1631, 1395.  $^1H$  NMR (free acid, DMSO- $d_6$ ):  $\delta$  10.32 (s, 1H), 8.65 (s, 1H), 8.09 (d,  $J = 8.00$  Hz, 1H), 7.29–7.17 (m, 5H), 4.45–4.34 (m, 1H), 3.09–2.99 (m, 1H), 2.86–2.75 (m, 1H), 2.04–1.86 (m, 4H), 1.37 (bs, 4H), 1.12–1.04 (m, 4H).  $^{13}C$  NMR (free acid, DMSO- $d_6$ ):  $\delta$  173.25, 172.16, 169.16, 137.78, 129.05, 128.10, 126.34, 53.29, 36.77, 35.06, 32.28, 28.39, 28.19, 25.11, 25.04. MS (EI):  $m/z$  336 ( $M^+$ ). Anal. ( $C_{29}H_{47}N_3O_5$ ) C, H, N.

**(S)-3-(4-Methoxy-phenyl)-2-(7-trityloxycarbamoyl-heptanoylamino)propionic Acid Methyl Ester (10j).** Compound **10j** was synthesized by method B from **9b** (500 mg, 1.16 mmol), NMM (0.128 mL, 117 mg, 1.16 mmol), isobutyl chloroformate (0.151 mL, 158 mg, 1.16 mmol), *O*-methyl-*L*-tyrosine methyl ester hydrochloride (285 mg, 1.16 mmol), and NMM (0.255 mL, 234 mg, 2.32 mmol). The crude product was chromatographed using ethyl acetate/hexane (3:1); yield 550 mg (75%); mp 146 °C. IR: 1750, 1669, 1644.  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  7.95 (bs, 1H), 7.41–7.72 (m, 15H), 7.17 (d,  $J = 8.7$  Hz, 2H), 6.99 (d,  $J = 8.8$  Hz, 2H), 6.03 (d,  $J = 8.0$  Hz, 1H), 4.98–5.05 (m, 1H), 3.95 (s, 3H), 3.89 (s, 3H), 3.16–3.29 (m, 2H), 2.26–2.34 (m, 2H), 1.013–1.812 (m, 10H). MS (EI):  $m/z$  243 ( $Trt^+$ ).

**(S)-3-(4-Methoxy-phenyl)-2-(7-hydroxycarbamoyl-heptanoylamino)propionic Acid Methyl Ester (11j).** Compound **11j** was synthesized by method E from **10j** (150 mg, 0.240 mmol); yield 85 mg (93%); mp 103 °C. IR: 1739, 1666.  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  10.48 (s, 1H), 8.80 (s, 1H), 8.35 (d,  $J = 7.8$  Hz, 1H), 7.32 (d,  $J = 8.5$  Hz, 2H), 7.03 (d,  $J = 8.6$  Hz, 2H), 4.65–4.58 (m, 1H), 3.91 (s, 3H), 3.79 (s, 3H), 3.18–3.12 (m, 1H), 3.04–2.96 (m, 1H), 2.24 (t,  $J = 7.4$  Hz, 2H), 2.12 (t,  $J = 7.5$  Hz, 2H), 1.66–1.57 (m, 4H), 1.40–1.32 (m, 4H).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  172.64, 158.84, 130.31, 128.00, 114.11, 112.42, 55.30, 53.17, 52.33, 37.18, 36.23, 28.57, 25.29. MS (EI):  $m/z$  380 ( $M^+$ ). Anal. ( $C_{19}H_{28}N_2O_6$ ) C, H, N.

**(S)-3-(4-Nitro-phenyl)-2-(7-trityloxycarbamoyl-heptanoylamino)propionic Acid Methyl Ester (10k).** Compound **10k** was synthesized by method B from **9b** (630 mg, 1.46 mmol), NMM (0.161 mL, 148 mg, 1.46 mmol), isobutyl chloroformate (0.191 mL, 199 mg, 1.46 mmol), *p*-nitro-*L*-phenylalanine methyl ester hydrochloride (381 mg, 1.46 mmol), and NMM (0.322 mL, 296 mg, 2.92 mmol). The crude product was chromatographed using ethyl acetate/hexane (1:2); yield 470 mg (50%); mp 166 °C. IR: 1748, 1646.  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  8.15 (d,  $J = 8.8$  Hz, 2H), 7.76 (bs, 1H), 7.45–7.27 (m, 17H), 6.09 (d,  $J = 6.9$  Hz, 1H), 4.97–4.87 (m, 1H), 3.74 (s, 3H), 3.24–3.10 (m, 2H), 2.17–2.09 (m, 2H), 1.72–1.44 (m, 4H), 1.30–1.15 (m, 6H).  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  173.10, 172.02, 147.59, 144.41, 141.58, 130.56, 129.43, 128.45, 124.03, 53.12, 52.95, 38.31, 36.59, 31.57, 28.97, 25.56. MS (EI):  $m/z$  165 ( $C_{13}H_9^+$ ).

**(S)-2-(7-Hydroxycarbamoyl-heptanoylamino)-3-(4-nitro-phenyl)propionic Acid Methyl Ester (11k).** Compound **11k** was synthesized by method E from **10k** (400 mg, 0.627

mmol); yield 156 mg (63%); mp 158 °C. IR: 1737, 1642. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.32 (s, 1H), 8.66 (s, 1H), 8.32 (d, *J* = 7.9 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 4.62–4.53 (m, 1H), 3.61 (s, 3H), 3.23–2.94 (m, 2H), 2.04–1.85 (m, 4H), 1.38–1.35 (m, 4H), 1.11 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.21, 171.65, 169.05, 146.28, 145.68, 130.40, 123.12, 52.58, 51.86, 36.28, 34.86, 32.15, 28.22, 28.05, 24.89. MS (EI): *m/z* 395 (M<sup>+</sup>). Anal. (C<sub>18</sub>H<sub>25</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

**(S)-2-(7-Benzylloxycarbonyl-heptanoylamino)-3-biphenyl-4-yl-propionic Acid Methyl Ester (10l).** Compound **10l** was synthesized by method B from **9a** (300 mg, 1.07 mmol), NMM (0.117 mL, 108 mg, 1.07 mmol), isobutyl chloroformate (0.140 mL, 146 mg, 1.07 mmol), l-biphenylalanine methyl ester hydrochloride (312 mg, 1.07 mmol), and NMM (0.234 mL, 216 mg, 1.07 mmol). The crude product was chromatographed using dichloromethane/methanol (25:1); yield 250 mg (45%); mp 143 °C. IR: 1739, 1654. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 7.59–7.26 (m, 14H), 4.88 (s, 2H), 4.76–4.69 (m, 1H), 3.71 (s, 3H), 3.26–3.16 (m, 1H), 3.02–2.90 (m, 1H), 2.14 (t, *J* = 7.3 Hz, 2H), 1.95 (t, *J* = 7.3 Hz, 2H), 1.66–1.33 (m, 4H), 1.33–0.97 (m, 4H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 176.07, 173.61, 142.04, 141.04, 137.40, 130.69, 130.29, 129.83, 78.93, 52.69, 38.05, 36.59, 33.62, 29.58, 26.53, 26.34. MS (EI): *m/z* 516 (M<sup>+</sup>).

**(S)-3-Biphenyl-4-yl-2-(7-hydroxycarbonyl-heptanoylamino)propionic Acid Methyl Ester (11l).** Compound **11l** was synthesized using method D from **10l** (200 mg, 0.390 mmol) and 10% Pd on charcoal (20 mg); yield 114 mg (69%); mp 136 °C. IR: 1739, 1647. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.31 (s, 1H), 8.65 (s, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 7.65–7.27 (m, 9H), 4.55–4.46 (m, 1H), 3.61 (s, 3H), 3.11–2.84 (m, 2H), 2.08–1.84 (m, 4H), 1.41–1.38 (m, 4H), 1.30–1.00 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.19, 172.05, 169.01, 139.77, 138.24, 136.55, 129.52, 128.78, 127.14, 126.36, 53.22, 51.67, 47.03, 36.21, 34.88, 32.13, 28.24, 28.09, 24.91. MS (EI): *m/z* 426 (M<sup>+</sup>). Anal. (C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub>) C, H, N.

**(S)-2-(7-Benzylloxycarbonyl-heptanoylamino)-3-methylbutyric Acid Methyl Ester (10m).** Compound **10m** was synthesized by method A from **9a** (300 mg, 1.07 mmol), DIPEA (0.185 mL, 138 mg, 1.07 mmol), BOP-Cl (272 mg, 1.07 mmol), L-valine methyl ester hydrochloride (198 mg, 1.18 mmol), and DIPEA (0.555 mL, 414 mg, 3.21 mmol); yield 270 mg (64%); mp 60 °C. IR: 1739, 1646, 1538. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.79 (bs, 1H), 7.37–7.26 (m, 5H), 6.07 (d, *J* = 8.69 Hz, 1H), 4.89 (s, 2H), 4.54 (dd, *J* = 8.64/4.93 Hz, 1H), 3.71 (s, 3H), 2.26–1.99 (m, 5H), 1.64–1.54 (m, 4H), 1.30–1.25 (m, 4H), 0.98–0.87 (m, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 173.13, 172.86, 129.24, 128.78, 128.67, 57.06, 52.15, 36.42, 31.30, 28.55, 25.40, 24.86, 24.77, 19.01, 17.96, 17.85. MS (EI): *m/z* 392 (M<sup>+</sup>).

**(S)-2-(7-Hydroxycarbonyl-heptanoylamino)-3-methylbutyric Acid Methyl Ester (11m).** Compound **11m** was synthesized by method D from **10m** (170 mg, 0.43 mmol) and 10% Pd on charcoal (17 mg); yield 77 mg (59%) viscous oil. IR: 1742, 1652. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.33 (s, 1H), 8.66 (s, 1H), 8.07 (d, *J* = 8.08 Hz, 1H), 4.17–4.10 (m, 1H), 3.60 (s, 3H), 2.16–1.87 (m, 5H), 1.45 (m, 4H), 1.20 (m, 4H), 0.87–0.82 (m, 6H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.72, 172.30, 169.12, 57.37, 51.58, 35.11, 34.86, 32.28, 29.80, 28.40, 25.25, 25.07, 19.01, 18.34. MS (EI): *m/z* 302 (M<sup>+</sup>), 72 (C<sub>4</sub>H<sub>10</sub>N<sup>+</sup>). Anal. (C<sub>14</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>·0.5H<sub>2</sub>O) C, H, N.

**(S)-2-(7-Benzylloxycarbonyl-heptanoylamino)-3-naphthalen-1-yl-propionic Acid Methyl Ester (10n).** Compound **10n** was synthesized by method B from **9a** (300 mg, 1.07 mmol), NMM (0.118 mL, 108 mg, 1.07 mmol), isobutyl chloroformate (0.140 mL, 146 mg, 1.07 mmol), β-(1-naphthyl)-L-alanine methyl ester hydrochloride (284 mg, 1.07 mmol), and NMM (0.236 mL, 216 mg, 2.14 mmol); yield 360 mg (69%); mp 80 °C. IR: 1744, 1648, 1539. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.58 (bs, 1H), 8.09–7.22 (m, 12H), 6.03 (d, *J* = 7.6 Hz, 1H), 5.03–4.93 (m, 1H), 4.89 (s, 2H), 3.62 (s, 3H), 3.58–3.51 (m, 2H), 2.11–1.99 (m, 4H), 1.61–1.47 (m, 4H), 1.39–1.04 (m, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 172.77, 172.61, 134.05, 132.05, 132.53, 132.37, 129.27, 128.97, 128.82, 128.71, 127.46, 126.44, 125.90, 125.34, 123.62, 78.47, 53.20, 52.31, 36.20, 35.27, 32.74, 28.46, 25.11, 24.77. MS (EI): *m/z* 490 (M<sup>+</sup>).

**(S)-2-(7-Hydroxycarbonyl-heptanoylamino)-3-naphthalen-1-yl-propionic Acid Methyl Ester (11n).** Compound **11n** was synthesized by method D from **10n** (300 mg, 0.45 mmol) and 10% Pd on charcoal (30 mg); yield 115 mg (64%); mp 148 °C. IR: 1722, 1641. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.33 (s, 1H), 8.67 (s, 1H), 8.38 (d, *J* = 7.7 Hz, 1H), 8.08–7.37 (m, 7H), 4.63–4.57 (m, 1H), 3.58 (s, 3H), 3.52–3.20 (m, 2H), 2.04–1.86 (m, 4H), 1.40–1.33 (m, 4H), 1.24–0.90 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.21, 172.12, 169.05, 133.33, 133.16, 131.29, 128.61, 127.25, 127.19, 126.14, 125.52, 123.06, 52.62, 51.73, 34.86, 33.95, 32.17, 28.24, 28.05, 24.87. MS (EI): *m/z* 400 (M<sup>+</sup>). Anal. (C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>) C, H, N.

**(S)-2-(7-Benzylloxycarbonyl-heptanoylamino)-3-naphthalen-2-yl-propionic Acid Methyl Ester (10o).** Compound **10o** was synthesized by method B from **9a** (300 mg, 1.07 mmol), NMM (0.118 mL, 108 mg, 1.07 mmol), isobutyl chloroformate (0.140 mL, 1.07 mmol), β-(2-naphthyl)-L-alanine methylester hydrochloride (284 mg, 1.07 mmol), and NMM (0.236 mL, 216 mg, 2.14 mmol); yield 450 mg (86%); mp 103 °C. IR: 3306, 1758, 1644, 1539. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.35 (bs, 1H), 7.19–7.83 (m, 12H), 5.93 (d, *J* = 7.8 Hz, 1H), 4.91–5.01 (m, 3H), 3.73 (s, 3H), 3.19–3.73 (m, 2H), 2.13 (t, *J* = 7.3 Hz, 2H), 1.97 (bs, 2H), 1.55 (bs, 4H), 1.21 (bs, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 172.74, 172.40, 133.46, 132.55, 129.27, 128.82, 128.69, 128.38, 128.09, 127.76, 127.61, 127.25, 126.32, 125.89, 78.21, 53.04, 53.043, 38.10, 36.21, 32.84, 28.30, 25.22, 24.87. MS (EI): *m/z* 490 (M<sup>+</sup>).

**(S)-2-(7-Hydroxycarbonyl-heptanoylamino)-3-naphthalen-2-yl-propionic Acid Methyl Ester (11o).** Compound **11o** was synthesized by method D from **10o** (400 mg, 0.82 mmol) and 10% Pd on charcoal (40 mg); yield 265 mg (81%); mp 124 °C. IR: 1739, 1646, 1539. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.25 (bs, 1H), 8.77 (bs, 1H), 8.18 (d, *J* = 8.1 Hz, 1H), 7.80–7.63 (m, 4H), 7.43–7.29 (m, 3H), 4.52 (m, 1H), 3.53 (s, 3H), 3.16–2.93 (m, 2H), 1.97–1.78 (m, 4H), 1.33–1.23 (m, 4H), 1.02–1.00 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.30, 172.25, 169.06, 135.04, 132.94, 131.87, 127.47, 127.41, 126.05, 125.56, 53.37, 51.87, 36.84, 34.97, 32.24, 28.33, 28.16, 25.07, 24.96. MS (EI): *m/z* 400 (M<sup>+</sup>). Anal. (C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>) C, H, N.

**(S)-3-Thiophen-2-yl-2-(7-trityloxycarbonyl-heptanoylamino)propionic Acid Methyl Ester (10p).** Compound **10p** was synthesized by method B from **9b** (610 mg, 1.41 mmol), NMM (0.155 mL, 143 mg, 1.41 mmol), isobutyl chloroformate (0.184 mL, 193 mg, 1.41 mmol), β-(2-thienyl)-L-alanine methyl ester hydrochloride (313 mg, 1.41 mmol), and NMM (0.310 mL, 286 mg, 2.82 mmol); yield 670 mg (79%); mp 130 °C. IR: 1747, 1651. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.74 (bs, 1H), 7.34–7.27 (m, 15H), 7.17 (d, *J* = 5.2 Hz, 1H), 6.95–6.91 (m, 1H), 6.76 (d, *J* = 3.0 Hz, 1H), 6.07 (d, *J* = 7.3 Hz, 1H), 4.93–4.84 (m, 1H), 3.76 (s, 3H), 3.38 (d, *J* = 4.8 Hz, 2H), 2.21–2.13 (m, 2H), 1.85–1.48 (m, 4H), 1.18–0.95 (m, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 172.62, 171.62, 137.46, 129.14, 128.16, 127.09, 126.78, 124.92, 53.01, 52.53, 36.50, 32.15, 28.84, 25.33. MS (EI): *m/z* 243 (Trt<sup>+</sup>), 165 (C<sub>13</sub>H<sub>9</sub><sup>+</sup>), 126 (C<sub>6</sub>H<sub>8</sub>NS<sup>+</sup>).

**(S)-2-(7-Hydroxycarbonyl-heptanoylamino)-3-thiophen-2-yl-propionic Acid Methyl Ester (11p).** Compound **11p** was synthesized by method E from **10p** (500 mg, 0.835 mmol); yield 120 mg (42%); mp 85 °C. IR: 1735, 1672. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.33 (s, 1H), 8.67 (s, 1H), 8.32 (d, *J* = 7.7 Hz, 1H), 7.34 (d, *J* = 4.9 Hz, 1H), 6.95–6.89 (m, 2H), 4.50–4.42 (m, 1H), 3.61 (s, 3H), 3.30–3.04 (m, 2H), 2.07 (t, *J* = 6.96 Hz, 2H), 1.91 (t, *J* = 7.14, 2H), 1.43 (bs, 4H), 1.17 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.29, 171.54, 169.08, 139.00, 126.72, 126.25, 124.65, 53.38, 51.84, 34.93, 32.20, 30.89, 28.29, 28.15, 24.94. MS (EI): *m/z* 356 (M<sup>+</sup>). Anal. (C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S) C, H, N.

**(S)-3-(1H-Indol-3-yl)-2-(7-trityloxycarbonyl-heptanoylamino)propionic Acid Methyl Ester (10q).** Compound **10q** was synthesized by method B from **9b** (500 mg, 1.16 mmol), NMM (0.128 mL, 117 mg, 1.16 mmol), isobutyl chloroformate (0.151 mL, 158 mg, 1.16 mmol), l-tryptophane methyl ester hydrochloride (293 mg, 1.16 mmol), and NMM (0.255 mL, 234 mg, 2.32 mmol). The crude product was chromatographed using ethyl acetate/hexane (2:1); yield 280 mg (39%); mp 112

°C. IR: 1740, 1657. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.67 (bs, 1H), 6.96–7.53 (m, 20H), 5.92 (d, *J* = 8.1 Hz, 1H), 4.91–4.95 (m, 1H), 3.72 (s, 3H), 3.30–3.34 (m, 2H), 2.09 (t, *J* = 1.9 Hz, 2H), 0.89–1.83 (m, 10H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 172.65, 129.11, 128.22, 123.03, 122.19, 119.61, 118.55, 111.49, 52.63, 52.46, 36.50, 32.90, 31.24, 28.71, 27.68, 25.35. MS (EI): *m/z* 165 (C<sub>13</sub>H<sub>9</sub><sup>+</sup>), 243 (Trt<sup>+</sup>).

**(S)-2-(7-Hydroxycarbamoyl-heptanoylamino)-3-(1H-indol-3-yl)propionic Acid Methyl Ester (11q).** Compound **11q** was synthesized by method E from **10q**; yield 110 mg (59%); mp 118 °C. IR: 1739, 1658, 1604, 1548. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.85 (s, 1H), 10.36 (s, 1H), 8.70 (s, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 6.96–7.49 (m, 5H), 4.46–4.49 (m, 1H), 3.56 (s, 3H), 3.00–3.45 (m, 2H), 1.87–2.08 (m, 4H), 1.38–1.42 (m, 4H), 1.11–1.14 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.69, 172.49, 169.32, 136.15, 127.10, 123.70, 121.60, 118.49, 118.06, 111.51, 109.65, 53.09, 51.86, 35.02, 32.33, 28.42, 28.31, 27.11, 25.11. MS (EI): *m/z* 253. Anal. (C<sub>20</sub>H<sub>27</sub>N<sub>3</sub>O<sub>5</sub>) C, H, N.

**(S)-Octanedioic Acid Benzyloxy-amide (2-Naphthalen-1-yl-1-phenethylcarbamoyl-ethyl)amide (10r).** Compound **10r** was synthesized by method B from **9a** (300 mg, 1.07 mmol), NMM (0.118 mL, 108 mg, 1.07 mmol), isobutyl chloroformate (0.140 mL, 146 mg, 1.07 mmol), (*S*)-2-amino-3-naphthalen-1-yl-*N*-phenethyl-propionamide tosylate (525 mg, 1.07 mmol), and NMM (0.236 mL, 216 mg, 2.14 mmol); yield 400 mg (67%); mp 130 °C. IR: 1639, 1546. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 6.95–8.08 (m, 17H), 4.73 (s, 2H), 4.62–4.60 (m, 1H), 3.39–3.46 (m, 1H), 3.07–3.35 (m, 3H), 2.55–2.40 (m, 2H), 2.04–1.88 (m, 4H), 1.43–1.29 (m, 4H), 1.15–0.95 (m, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 175.85, 173.41, 140.30, 135.42, 134.42, 133.38, 130.31, 129.85, 129.76, 129.61, 129.45, 128.69, 127.30, 127.25, 126.70, 126.37, 124.74, 78.90, 55.55, 41.90, 36.70, 36.32, 33.64, 29.58, 26.53, 26.36. MS (EI): *m/z* 302 (C<sub>21</sub>H<sub>20</sub>NO<sup>+</sup>).

**(S)-Octanedioic Acid Hydroxyamide (2-Naphthalen-1-yl-1-phenethylcarbamoyl-ethyl)amide (11r).** Compound **11r** was synthesized by method D from **10r** (320 mg, 0.580 mmol) and 10% Pd on charcoal (32 mg); yield 155 mg (54%); mp 137 °C. IR: 1640, 1543, 1456. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 10.44 (s, 1H), 8.76 (s, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 8.19–7.29 (m, 12H), 4.77–4.69 (m, 1H), 3.62–3.26 (m, 2H), 2.76–2.85 (m, 2H), 2.04–2.20 (m, 4H), 1.45–1.59 (m, 4H), 1.20–1.32 (m, 4H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 172.37, 171.37, 169.48, 139.73, 134.27, 133.73, 132.04, 128.97, 128.88, 128.61, 127.70, 127.31, 126.37, 125.84, 125.60, 124.18, 53.74, 35.53, 35.43, 35.35, 32.64, 28.73, 28.60, 25.36. MS (EI): *m/z* 489 (M<sup>+</sup>). Anal. (C<sub>29</sub>H<sub>35</sub>N<sub>3</sub>O<sub>4</sub>·0.5H<sub>2</sub>O) C, H, N.

**Analysis of the Optical Purity of 11d,e.** A Chirex 3014 column (Phenomenex, 250 mm × 4.6 mm) was used for the determination of enantiomeric purity. Stock solutions of the compounds (5 mg/mL in methanol) were diluted with the chromatography eluent (isohexane/dichloromethane/methanol, 60/40/7.5, v/v). Retention time was 8.96 min for **11e** and 9.76 min for **11d**.

**Enzyme Inhibition.** For partial purification of maize HD-1, maize embryos were extracted and a crude chromatin preparation was employed.<sup>46</sup> The chromatin extract was applied to Q-Sepharose chromatography, and HD-1 enzyme fractions were pooled and concentrated by ammonium sulfate precipitation (20–45%). The precipitate was subjected to Sephacryl Hi-Prep S-200, and peak fractions were used for HDAC inhibition assays.<sup>39</sup> For purification of maize HD-2, the chromatin fraction of maize embryos was separated on Q-sepharose.<sup>36</sup> Radioactively labeled chicken core histones are used as the enzyme substrate according to established methodology.<sup>47</sup> The enzymes are liberating tritiated acetic acid from the substrate, which is quantitated by scintillation counting. IC<sub>50</sub> values are results of triple determinations. An amount of 50 μL of maize enzyme (at 30 °C) was incubated (30 min) with 10 μL of total [<sup>3</sup>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 it was centrifuged (10 000g, 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.

Rat liver HDAC was purified on Q-sepharose with an increasing gradient of sodium chloride. The detailed procedure is described elsewhere,<sup>37</sup> and this preparation is commercially available as well (Calbiochem). Our nonisotopic coumarin-labeled acetyllysine, which can be obtained from Calbiochem (Nr. 382155), was employed as a substrate.<sup>38</sup> We use a modification of the original procedure that involves the use of an internal standard in order to ensure accuracy and precision.<sup>48</sup> The latter method was slightly modified with an increased starting concentration of the substrate, which leads to higher reproducibility of the IC<sub>50</sub> values (data not shown). In both assays, **2** and **6a,c** were used as reference compounds while blank solvents and the O-benzyl hydroxamate precursor of **5<sup>35</sup>** served as negative controls. The HPLC assay was performed on the LiChrosorb column with acetonitrile/water (40/60, v/v) as mobile phase at a flow rate of 0.6 mL/min. Retention time of substrate is 3.47 min, and that of the internal standard 7-hydroxycoumarin is 2.17 min. Excitation wavelength is 330 nm, and emission wavelength is 395 nm. Results are taken from duplicate determinations on six levels of inhibitor concentration.

Stock solutions of the inhibitors were made at a concentration of 12 mM in DMSO and 1 mg/mL in ethanol for TSA and were further diluted with enzyme buffer (15 mM tris-HCl, pH 7.9; 0.25 mM EDTA; 10 mM NaCl; 10% (v/v) glycerol; 10 mM 2-mercaptoethanol). A substrate stock solution was prepared using an aliquot of 12 μL of a solution of the fluorogenic substrate (4.682 mg/mL in ethanol), 24 μL of a solution of the standard 7-hydroxycoumarin (3.66 mg/mL in DMSO), and enzyme buffer to a total volume of 1 mL.

A 10 μL amount of the substrate/standard stock solution was added to a mixture of 100 μL of rat enzyme preparation (at 4 °C) and 10 μL of inhibitor dilution. After 15 min at 4 °C, the mixture was then incubated for 90 min at 37 °C. After this time, the reaction was stopped by the addition of 72 μL of 1 M HCl/0.4 M sodium acetate and 800 μL of ethyl acetate. After it was centrifuged (10 000g, 5 min), an aliquot of 200 μL of the upper phase was taken and the solvent was removed by a stream of nitrogen. The residue was dissolved in 600 μL of the chromatography eluent, and 20 μL was injected via autosampler onto the HPLC system. The amount of remaining substrate is calculated relative to the substrate control without enzyme (each as quotient of the peak area of the substrate divided by the peak area of the internal standard).

**Induction of Terminal Cell Differentiation.** Friend leukemic cells (MEL DS19 murine erythroleukemia cells) were maintained in DMEM 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<sub>2</sub> 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 DMEM/well. If compounds were dissolved in DMSO, control wells contained the same amount of solvent (generally 2 μL/mL medium, 0.1% final concentration). Subsequently, the cell suspension was added to the wells (1 mL/well, 8 × 10<sup>4</sup> cells/mL; final cell concentration 4 × 10<sup>4</sup> 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 Friend leukemic cells accumulate hemoglobin. Therefore, the induction of cell differentiation was determined by benzidine staining according to the literature.<sup>49</sup> To 100 μL of cells suspension, 10 μL of a 0.4% solution of benzidine in 12% acetic acid containing 2% H<sub>2</sub>O<sub>2</sub> was added. Within 5 min, hemoglobin-containing cells stain 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 profiles, a range of concentration was chosen for a dose–response analysis. In selected cases, dose–response experiments were repeated at the same concentrations and deviations were below 5%. So, we have not included error bars and the figures are representations of one experiment and not a mean. Trichostatin A (**2**) and SAHA (**6a**), known inducers of cell differentiation in Friend leukemic cells, were included as positive controls.

**Histone Hyperacetylation.** Friend leukemic cells ( $1 \times 10^5$  cells/ml in 20 mL) were incubated in DMEM for 24 h and then treated with  $5 \mu\text{M}$  **111** for various time periods as indicated. Histones were isolated as described previously<sup>50</sup> with slight modifications. Briefly,  $5 \times 10^6$  cells were harvested by centrifugation and washed twice with cold phosphate-buffered saline (PBS). Cells were lysed with 1 mL of lysis buffer (10 mM tris HCl, 50 mM sodium bisulfite, 1% Triton X-100, 10 mM MgCl<sub>2</sub>, 8.6% sucrose, pH 6.5), and nuclei were collected by centrifugation. Cell pellets were washed with 10 mM tris HCl, pH 7.4, containing 13 mM EDTA and suspended in 0.1 mL of ice-cold water. HCl was added until a final concentration of 0.4 M, and the mixtures were incubated on ice for 1 h. Acid soluble nuclear proteins were obtained by centrifugation at 10 000g for 10 min. Supernatants were mixed with 300  $\mu\text{L}$  of cold ethanol and incubated at  $-20^\circ\text{C}$  overnight. Histone-containing pellets were obtained by centrifugation, air-dried, and dissolved in 50  $\mu\text{L}$  of water. Protein was quantified according to Bradford.<sup>51</sup> The samples were subjected to AUT-PAGE analysis as described before.<sup>43,44</sup>

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**Supporting Information Available:** Experimental and spectral data for amino acid building blocks and calibration curves and HPLC chromatograms for chiral separation of **10d.e**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- Meinke, P. T.; Liberator, P. Histone deacetylase: a target for antiproliferative and antiprotozoal agents. *Curr. Med. Chem.* **2001**, *8*, 211–235.
- Jung, M. Inhibitors of histone deacetylase as new anticancer agents. *Curr. Med. Chem.* **2001**, *8*, 1505–1511.
- Weidle, U. H.; Grossmann, A. Inhibition of histone deacetylases: a new strategy to target epigenetic modifications for anticancer treatment. *Anticancer Res.* **2000**, *20*, 1471–1485.
- Torchia, J.; Glass, C.; Rosenfeld, M. G. Co-activators and co-repressors in the integration of transcriptional responses. *Curr. Opin. Cell Biol.* **1998**, *10*, 373–383.
- Cress, W. D.; Seto, E. Histone deacetylases, transcriptional control, and cancer. *J. Cell Physiol.* **2000**, *184*, 1–16.
- Archer, S. Y.; Meng, S.; Shei, A.; Hodin, R. A. p21(WAF1) is required for butyrate-mediated growth inhibition of human colon cancer cells. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6791–6796.
- Sambucetti, L. C.; Fischer, D. D.; Zabudoff, S.; Kwon, P. O.; Chamberlin, H.; Trogani, N.; Xu, H.; Cohen, D. Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. *J. Biol. Chem.* **1999**, *274*, 34940–34947.
- Redner, R. L.; Wang, J.; Liu, J. M. Chromatin remodeling and leukemia: new therapeutic paradigms. *Blood* **1999**, *94*, 417–428.
- Mahlknecht, U.; Hoelzer, D. Histone acetylation modifiers in the pathogenesis of malignant disease. *Mol. Med.* **2000**, *6*, 623–644.
- Jung, M. Histone Deacetylases. In *Transcription Factors and Other Nuclear Proteins as Targets for Cancer Chemotherapy*; La Thangue, N. B., Bandara, L. R., Eds.; Humana Press: Totowa, 2002; pp 123–144.
- Coffey, D. C.; Kutko, M. C.; Glick, R. D.; Butler, L. M.; Heller, G.; Rifkind, R. A.; Marks, P. A.; Richon, V. M.; La Quaglia, M. P. The histone deacetylase inhibitor, CBHA, inhibits growth of human neuroblastoma xenografts in vivo, alone and synergistically with all-trans retinoic acid. *Cancer Res.* **2001**, *61*, 3591–3594.
- Warrell, R. P., Jr.; He, L. Z.; Richon, V.; Calleja, E.; Pandolfi, P. P. Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. *J. Natl. Cancer Inst.* **1998**, *90*, 1621–1625.
- Yoshida, M.; Horinouchi, S.; Beppu, T. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *Bioessays* **1995**, *17*, 423–430.
- Jung, M.; Hoffmann, K.; Brosch, G.; Loidl, P. Analogues of trichostatin A and trapoxin B as histone deacetylase inhibitors. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1655–1658.
- Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3003–3007.
- Butler, L. M.; Webb, Y.; Agus, D. B.; Higgins, B.; Tolentino, T. R.; Kutko, M. C.; LaQuaglia, M. P.; Drobnjak, M.; Cordon-Cardo, C.; Scher, H. I.; Breslow, R.; Richon, V. M.; Rifkind, R. A.; Marks, P. A. Inhibition of transformed cell growth and induction of cellular differentiation by pyroxamide, an inhibitor of histone deacetylase. *Clin. Cancer Res.* **2001**, *7*, 962–970.
- Richon, V. M.; Webb, Y.; Merger, R.; Sheppard, T.; Jursic, B.; Ngo, L.; Civoli, F.; Breslow, R.; Rifkind, R. A.; Marks, P. A. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5705–5708.
- Jung, M.; Brosch, G.; Kölle, D.; Scherf, H.; Gerhäuser, C.; Loidl, P. Amide analogues of trichostatin A as inhibitors of histone deacetylase and inducers of terminal cell differentiation. *J. Med. Chem.* **1999**, *42*, 4669–4679.
- Massa, S.; Mai, A.; Sbardella, G.; Esposito, M.; Ragno, R.; Loidl, P.; Brosch, G. 3-(4-Aroyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamides, a new class of synthetic histone deacetylase inhibitors. *J. Med. Chem.* **2001**, *44*, 2069–2072.
- Suzuki, T.; Ando, T.; Tsuchiya, K.; Fukazawa, N.; Saito, A.; Mariko, Y.; Yamashita, T.; Nakanishi, O. Synthesis and histone deacetylase inhibitory activity of new benzamide derivatives. *J. Med. Chem.* **1999**, *42*, 3001–3003.
- Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, T.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4592–4597.
- Colletti, S. L.; Li, C.; Fisher, M. H.; Wyvratt, M. J.; Meinke, P. T. Tryptophan-replacement and indole-modified apicidins: synthesis of potent and selective antiprotozoal agents. *Tetrahedron Lett.* **2000**, *41*, 7825–7829.
- Meinke, P. T.; Coletti, S. L.; Ayer, M. B.; Darkin-Rattray, S. J.; Myers, R. M.; Schmatz, D. M.; Wyvratt, M. J.; Fisher, M. H. Synthesis of side chain modified apicidin derivatives: potent mechanism-based histone deacetylase inhibitors. *Tetrahedron Lett.* **2000**, *41*, 7831–7835.
- Colletti, S. L.; Myers, R. W.; Darkin-Rattray, S. J.; Schmatz, D. M.; Fisher, M. H.; Wyvratt, M. J.; Meinke, P. T. Design and synthesis of histone deacetylase inhibitors: the development of apicidin transition state analogues. *Tetrahedron Lett.* **2000**, *41*, 7837–7841.
- Meinke, P. T.; Colletti, S. L.; Doss, G.; Myers, R. W.; Gurnett, A. M.; Dulski, P. M.; Darkin-Rattray, S. J.; Allocco, J. J.; Galuska, S.; Schmatz, D. M.; Wyvratt, M. J.; Fisher, M. H. Synthesis of apicidin-derived quinolone derivatives: parasite-selective histone deacetylase inhibitors and antiproliferative agents. *J. Med. Chem.* **2000**, *43*, 4919–4922.
- Colletti, S. L.; Myers, R. W.; Darkin-Rattray, S. J.; Gurnett, A. M.; Dulski, P. M.; Galuska, S.; Allocco, J. J.; Ayer, M. B.; Li, C.; Lim, J.; Crumley, T. M.; Cannova, C.; Schmatz, D. M.; Wyvratt, M. J.; Fisher, M. H.; Meinke, P. T. Broad spectrum antiprotozoal agents that inhibit histone deacetylase: structure–activity relationships of apicidin. Part 1. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 107–111.
- Colletti, S. L.; Myers, R. W.; Darkin-Rattray, S. J.; Gurnett, A. M.; Dulski, P. M.; Galuska, S.; Allocco, J. J.; Ayer, M. B.; Li, C.; Lim, J.; Crumley, T. M.; Cannova, C.; Schmatz, D. M.; Wyvratt, M. J.; Fisher, M. H.; Meinke, P. T. Broad spectrum antiprotozoal agents that inhibit histone deacetylase: structure–activity relationships of apicidin. Part 2. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 113–117.

- (28) Sternson, S. M.; Wong, J. C.; Grozinger, C. M.; Schreiber, S. L. Synthesis of 7200 small molecules based on a substructural analysis of the histone deacetylase inhibitors trichostatin and trapoxin. *Org. Lett.* **2001**, *3*, 4239–4242.
- (29) Furumai, R.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Yoshida, M.; Horinouchi, S. Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 87–92.
- (30) 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.
- (31) Finnin, M. S.; Donigian, J. R.; Pavletich, N. P. Structure of the histone deacetylase SIRT2. *Nat. Struct. Biol.* **2001**, *8*, 621–625.
- (32) Min, J.; Landry, J.; Sternglanz, R.; Xu, R. M. Crystal structure of a SIR2 homolog-NAD complex. *Cell* **2001**, *105*, 269–279.
- (33) Lusser, A.; Brosch, G.; Loidl, A.; Haas, H.; Loidl, P. Identification of maize histone deacetylase HD2 as an acidic nucleolar phosphoprotein. *Science* **1997**, *277*, 88–91.
- (34) Gray, S. G.; Ekstrom, T. J. The human histone deacetylase family. *Exp. Cell Res.* **2001**, *262*, 75–83.
- (35) Schmidt, K.; Gust, R.; Jung, M. Inhibitors of histone deacetylase suppress the growth of MCF-7 breast cancer cells. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 353–357.
- (36) Brosch, G.; Lusser, A.; Goralik Schramel, M.; Loidl, P. Purification and characterization of a high molecular weight histone deacetylase complex (HD2) of maize embryos. *Biochemistry* **1996**, *35*, 15907–15914.
- (37) Hoffmann, K.; Brosch, G.; Loidl, P.; Jung, M. First nonradioactive assay for in vitro screening of histone deacetylase inhibitors. *Pharmazie* **2000**, *55*, 601–606.
- (38) Hoffmann, K.; Brosch, G.; Loidl, P.; Jung, M. A nonisotopic assay for histone deacetylase activity. *Nucleic Acids Res.* **1999**, *27*, 2057–2058.
- (39) Lechner, T.; Lusser, A.; Pipal, A.; Brosch, G.; Loidl, A.; Goralik-Schramel, M.; Sendra, R.; Wegener, S.; Walton, J. D.; Loidl, P. RPD3-type histone deacetylases in maize embryos. *Biochemistry* **2000**, *39*, 1683–1692.
- (40) Yoshida, M.; Nomura, S.; Beppu, T. Effects of trichostatins on differentiation of murine erythroleukemia cells. *Cancer Res.* **1987**, *47*, 3688–3691.
- (41) Marks, P. A.; Richon, V. M.; Kiyokawa, H.; Rifkind, R. A. Inducing differentiation of transformed cells with hybrid polar compounds: a cell cycle-dependent process. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10251–10254.
- (42) Lea, M. A.; Randolph, V. M.; Hodge, S. K. Induction of histone acetylation and growth regulation in erythroleukemia cells by 4-phenylbutyrate and structural analogues. *Anticancer Res.* **1999**, *19*, 1971–1976.
- (43) Alfageme, C. R.; Zweidler, A.; Mahowald, A.; Cohen, L. H. Histones of *Drosophila* embryos. Electrophoretic isolation and structural studies. *J. Biol. Chem.* **1974**, *249*, 3729–3736.
- (44) Kölle, D.; Brosch, G.; Lechner, T.; Lusser, A.; Loidl, P. Biochemical methods for analysis of histone deacetylases. *Methods* **1998**, *15*, 323–331.
- (45) Remiszewski, S. W.; Sambucetti, L. C.; Atadja, P.; Bair, K. W.; Cornell, W. D.; Green, M. A.; Howell, K. L.; Jung, M.; Kwon, P.; Trogani, N.; Walker, H. Inhibitors of human histone deacetylase: synthesis, enzyme and cellular activity of straight chain hydroxamates. *J. Med. Chem.* **2002**, *45*, 753–757.
- (46) Steinmüller, K.; Apel, K. A simple and efficient procedure for isolating plant chromatin which is suitable for studies of DNase I-sensitive domains and hypersensitive sites. *Plant Mol. Biol.* **1986**, *7*, 87–94.
- (47) Lechner, T.; Lusser, A.; Brosch, G.; Eberharter, A.; Goralik Schramel, M.; Loidl, P. A comparative study of histone deacetylases of plant, fungal and vertebrate cells. *Biochim. Biophys. Acta* **1996**, *1296*, 181–188.
- (48) Hoffmann, K.; Heltweg, B.; Jung, M. Improvement and validation of the fluorescence-based histone deacetylase assay using an internal standard. *Arch. Pharm. Pharm. Med. Chem.* **2001**, *334*, 248–252.
- (49) Richon, V. M.; Rifkind, R. A.; Marks, P. A. Differentiation of murine erythroleukemia cells (Friend Leukemia cells). In *Cell Biology: A Laboratory Handbook*; Academic Press Inc., New York, 1994; pp 213–217.
- (50) Huang, N.; Katz, J. P.; Martin, D. R.; Wu, G. D. Inhibition of IL-8 gene expression in Caco-2 cells by compounds which induce histone hyperacetylation. *Cytokine* **1997**, *9*, 27–36.
- (51) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
- (52) Heltweg, B.; Jung, M. A micro plate reader based nonisotopic histone deacetylase activity assay. *Anal. Biochem.* **2002**, *302*, 175–183.

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