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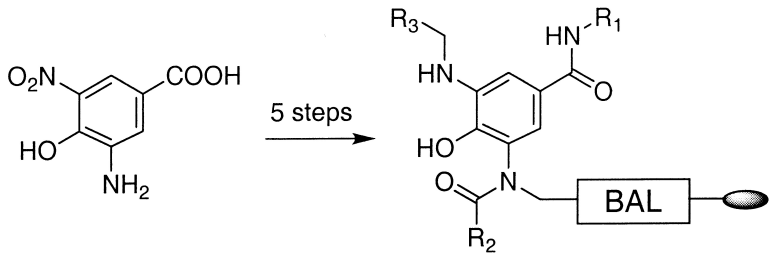

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Combinatorial Solid-Phase Synthesis of Hapalosin Mimetics[1,2]

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The solid-phase synthesis of a small library of mimetics of the cyclic depsipeptide hapalosin is described. 3-Amino-4-hydroxy-5-nitrobenzoic acid was anchored through the anilino moiety to a backbone amide linker (BAL) handle support. Using chemoselective reactions and without the need for protecting group manipulations, the benzoic acid group was first amidated, then the aniline nitrogen was acylated, and finally the nitro group was reduced to an amine and acylated or reductively alkylated, to generate a 12-member library.

Introduction

Although short-term treatments of cancer with antineoplastic chemotherapeutics are becoming increasingly effective, prolonged treatments frequently are hampered by the development of multidrug resistance (MDR). MDR is directed not only toward the actual drug in use but also to other structurally unrelated drugs. The development of MDR arises from at least two different proteins, including the 170 kDa transmembrane protein P-glycoprotein (P-gp, an ATP-dependent drug efflux pump) and human multidrug resistance-associated protein, MRP (a 180−195 kDa plasma membrane drug efflux pump). Compounds that inhibit the drug efflux pump of P-gp and/or MRP could serve as templates for design of potentially useful anticancer drugs to be used in combination with established cytopharmaca. The mechanism of action is yet unclear and can arise from various effects such as calcium channel blocking, interference with calmodulin action, blocking of the ATP-binding site, or perturbation of the cell membrane. Several compounds including verapamil, azidopine, nimipidine, and nifedipine have been found to inhibit P-gp, but although several MDR-reversing agents have entered clinical trials, none have yet been introduced successfully as routine treatment in cancer chemotherapy.

In 1994, Moore and co-workers reported the finding of hapalosin, **1**, a 12-membered cyclic depsipeptide from the lipophilic extracts of two strains of blue-green algae *Hapalosiphon welwitschii* W. & G. S. West (Stignonemataceae) with MDR-reversing activity (Scheme 1).[3] Compared to verapamil which is the standard among MDR modulators, hapalosin is equally effective in causing cell death and promoting accumulation of cytopharmaca such as vinblastine or Taxol. Thus, hapalosin, which itself is slightly cytotoxic, is a potential target for drug design aimed at MDR.

Since the discovery of hapalosin, several total syntheses of both hapalosin and analogues have been reported in the literature.[4−7] Due to the structural complexity of hapalosin, its structure is not readily adapted to exploration by solid-phase synthesis and combinatorial library generation. The use of scaffold structures represents an alternative in the pursuit for biologically active structures. Hirschmann, Nicolaou, Smith, and co-workers designed and synthesized biologically active somatostatin agonist mimetics based on a nonpeptidic D-glucopyranoside scaffold.[8] Along these lines, Armstrong et al. used a D-glucopyranoside scaffold in the design and synthesis of hapalosin mimetics, giving analogues with activity similar to hapalosin with respect to antagonizing MRP-mediated MDR, although none possessed the efficiency of hapalosin to reverse P-gp-mediated MDR.[9] The authors attributed this to the fact that the glucose scaffold is less flexible than hapalosin, and although the mimetics are able to adopt some of the main conformations of hapalosin, it might not fold into the actual bioactive conformation.[9] Therefore, to explore alternative scaffolds for hapalosin mimetics, we have investigated the use of a novel aromatic scaffold for solid-phase combinatorial synthesis of a 12-member library.

Results and Discussion

Previous studies on hapalosin mimetics[9,10] and simple model building revealed that functionalization of the scaffold should include branched aliphatic groups, an aromatic moiety, and long-chain aliphatic groups. Moreover, a polar group positioned close to the scaffold seems to be important for activity.[6] Some degree of flexibility as provided by, e.g., aromatic amides was essential in our choice of scaffold structure. Likewise, previous scaffolds have mainly been based on carbohydrate- or cyclohexane-based chairlike conformations with rather high polarity and not exploiting the potentially stronger fit from an nonpolar aromatic multifunctional scaffold. Therefore, we decided on an aromatic scaffold with one hydroxy group, two amino groups for subsequent N-acylation or reductive alkylation, and one carboxyl group for amidation, all spatially displayed in the scaffold approximately as in hapalosin. Furthermore, we wanted to avoid the use of protecting groups to minimize the number of solution steps for the preparation of the scaffold and for the subsequent solid-phase synthesis. We decided on a highly substituted benzene ring, 3-amino-4-

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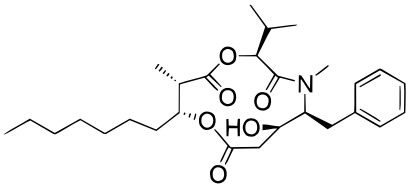
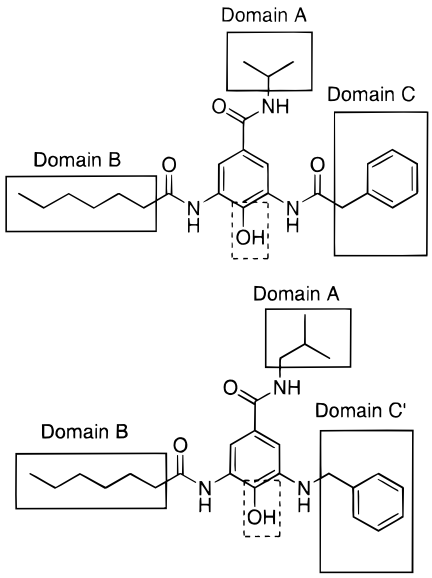
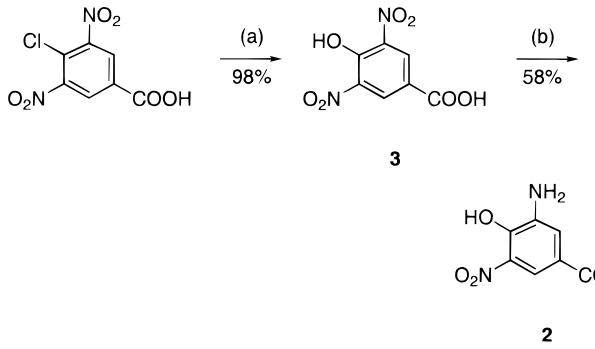
Scheme 1. Structure of Template Structure **1**, Hapalosin, and the Two Mimetic Structures Displaying the Three Main Domains A−C and the Polar Hydroxy Group



1
Hapalosin



Scheme 2[a]



3

2

[a] Reagents and conditions: (a) NaOH, H_2O, 18 h, then Aq HCl; (b) H_2, Pd/C, 18 h.

hydroxy-5-nitrobenzoic acid, **2**,[11] with the nitro group serving as a masked amine (Scheme 1).[12] The recently developed backbone amide linker (BAL) handle relies on reductive amination to anchor an amine containing compound to a solid support.[13,14] Aliphatic amines can, after N-acylation, be released by treatment with concentrated trifluoroacetic acid (TFA), whereas anilines can be released without prior acylation. In contrast to attachment through a carboxyl or hydroxy function, BAL anchoring does *not* occupy a functional group. Anchoring of **2** through an anilino group to a BAL handle would thus leave this nitrogen available for further derivatization.

Following a literature procedure, treatment of commercially available 4-chloro-3,5-dinitrobenzoic acid with aqueous base gave 4-hydroxy-3,5-dinitrobenzoic acid, **3** (Scheme 2). The selective reduction of only one of the nitro groups with ammonium hydrogen sulfide to give 3-amino-4-hydroxy-5-nitrobenzoic acid, **2**, has been reported; reduction with hydrogen over Raney nickel was reported to give only low yields.[11a] However, we found that the more benign and less malodorous hydrogenation over Pd/C at 1 bar in methanol in the *absence* of acid also effected monoreduction to yield **2**. In contrast hereto, when the reduction was performed in the *presence* of HCl, the diamino derivative was observed together with **2**.[15]

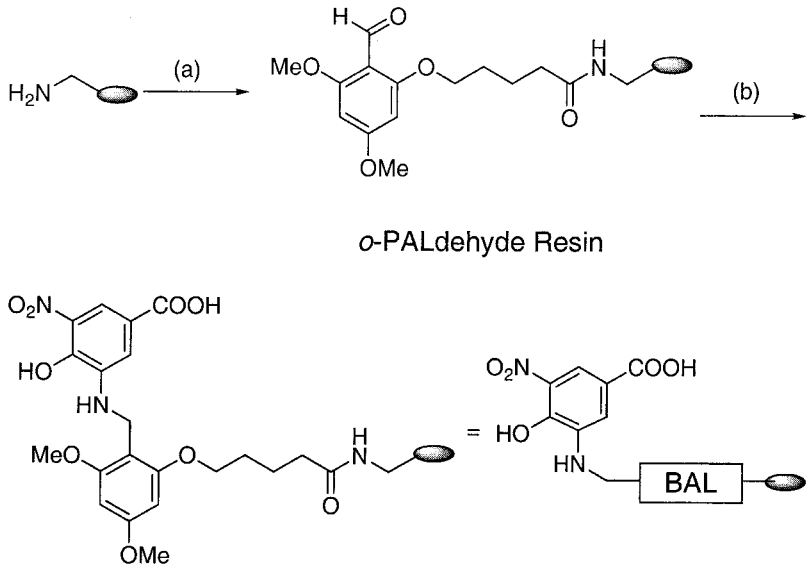
Solid-phase synthesis commenced with the PyBOP promoted coupling of PALdehyde[16] in DMF in the presence of *N*,*N*-diisopropylethylamine (DIEA) to a high-loading (1.0 mmol/g) polystyrene (PS) support (Scheme 3). The $NaBH_3CN$-mediated reductive amination of the PALdehyde support with derivative **2** in DMF−HOAc (99:1) gave the BAL anchored anilino derivative. As expected, the anilino-linked scaffold could be released from the BAL support by treatment with TFA−water (19:1). The loading was determined to be 0.65 mmol/g by analytical cleavage followed by HPLC analysis and comparison to a standard curve for **2**. In the following, after each step in model studies and on select samples in library syntheses, cleavages of small amounts of resin followed by analysis with HPLC and LC/MS were carried out. Final library products were cleaved off the resin, purified by column chromatography or preparative TLC, and characterized by MS and NMR.

Model Studies

In model studies toward the solid-phase synthesis of hapalosin mimetics, we first studied the amidation of the benzoic acid functionality (Scheme 4, step a). Activation with HATU/HOAt and reaction with 2-aminopropane gave the desired amide as well as an unidentified byproduct (*m*/*z* 295). Variation in equivalents of base, reaction time, and HOAt did not lead to any significant minimization of byproduct formation; however, substituting HATU/HOAt for PyBOP in the presence of DIEA without preactivation yielded the desired product while preventing formation of the byproduct.

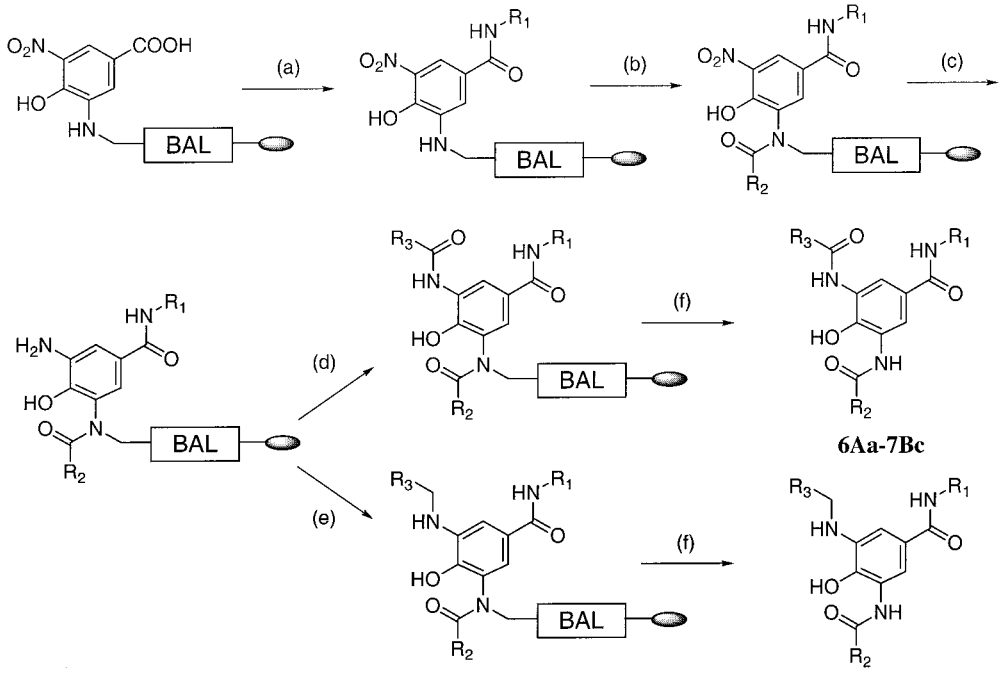
Analysis of the products after acylation of the first anilino amine with hexanoyl chloride (Scheme 4, step b) gave, according to HPLC, two compounds, tentatively assigned as the monoacylated (∼85−95%) and diacylated products (∼5−15%) with no starting material left. O-Deacylation of the acylated resin-bound products with 1 M sodium meth-

Scheme 3[a]



[a] Reagents and conditions: (a) *o*-PALdehyde, PyBOP, rt, 16 h; (b) **2**, $NaBH_3CN$, DMF−HOAc (99:1), 48 h.

Scheme 4[a]



[a] Reagents and conditions: (a) R_1NH_2, PyBOP, DIEA, DMF, rt, 3 h; (b) R_2COCl, DIEA, DCM, rt, 2 h; (c) $SnCl_2 \cdot 2H_2O$, DMF, rt, 40 min; (d) R_3COCl, DIEA, DCM, rt, 2 h; (e) benzaldehyde, $NaBH_3CN$, DMF−HOAc (99:1), rt, 10 h; (f) TFA−H_2O (19:1), rt, 2 h.

oxide in methanol for 30 min yielded the expected N-acylated product as well as the starting material (∼15%). Treatment with *n*-butylamine−DMF (1:9) for 1 min gave the same result. Most likely, the major components by HPLC analysis after acylation consist of mono N- and O-acylated products due to the low nucleophilicity and steric hindrance of the secondary aniline nitrogen. Furthermore, treatment with methoxide overnight did not yield more starting material, thus supporting the view that reformation of starting material was *not* due to methoxide promoted cleavage of the amide.[17] Attemps to optimize the N-acylation step further proved unsuccessful.

Following literature precedents for on-resin reduction of aromatic nitro groups,[18] treatment with freshly prepared 2 M $SnCl_2 \cdot 2H_2O$ in DMF smoothly reduced the nitro group to the corresponding amine with no significant byproducts observed (Scheme 4, step c). The second N-acylation was carried out with benzoyl chloride; the acylation was repeated to ensure full conversion to the expected benzamide (Scheme 4, step d).

Reductive alkylation with benzaldehyde (Scheme 4, step e) was carried with $NaBH_3CN$ in DMF−HOAc (99:1). However, a byproduct (at a 12% level) was observed, tentatively assigned the bis-alkylated product. Lowering the

equivalents of amine and $NaBH_3CN$, changing the reaction time, or substituting DMF−HOAc (99:1) for THF−HOAc (99:1) did not improve the product distribution. Finally, treatment with TFA−water (19:1) released the completed product into solution (Scheme 4, step f).

Library Design

To demonstrate the feasibility of this strategy for library synthesis, we designed a small 12-member library originating from 2 × 3 × 2 building blocks. For the first combinatorial step, amidation of the benzoic acid moiety, we chose two branched aliphatic groups, 2-aminopropane and 2-methyl-1-aminopropane. For the second step, which introduced long-chain aliphatic groups, the amine was N-acylated with *n*-hexanoyl or *n*-heptanoyl chloride. For the third step, the nitro group was reduced to an amine upon which aromatic groups were introduced by N-acylation with benzoyl chloride and phenacetyl chloride or by reductive alkylation with benzaldehyde.

Library Synthesis

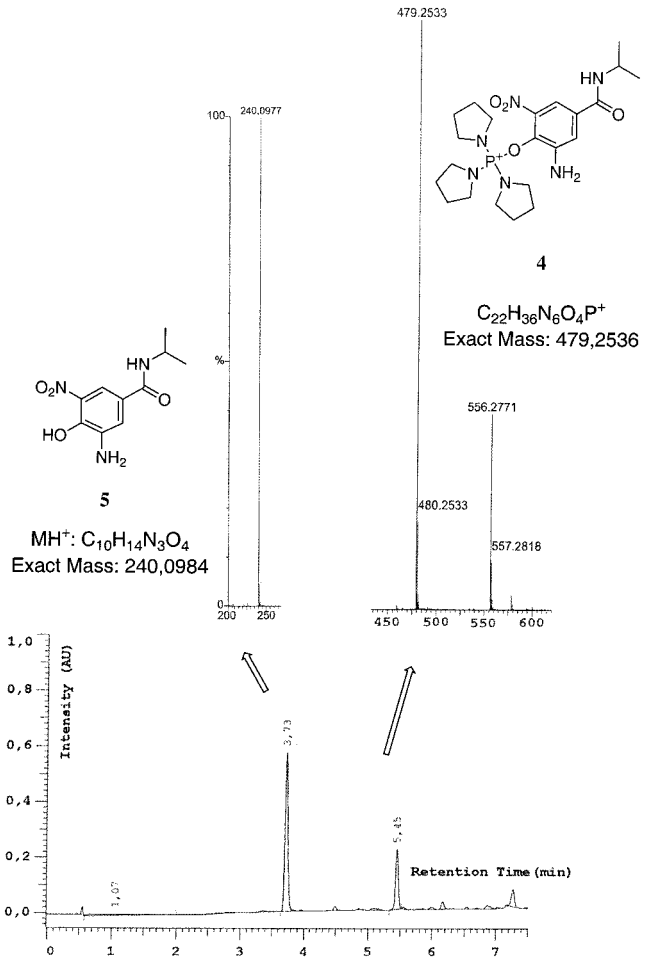
1. Solid-Phase Derivatization Step. In the first solid-phase derivatization step, the benzoic acid moiety was activated by PyBOP and amidated with 2-aminopropane or 2-methyl-1-aminopropane in DMF. In model studies, PyBOP was the reagent of choice (vide supra); however, in the library synthesis a new byproduct was obtained upon PyBOP activation, **4**,[19] together with expected 3-amino-4-hydroxy-5-nitro-*N*-isopropylbenzamide, **5** (Scheme 5). After HPLC−HRMS analysis, byproduct **4** was tentatively identified as a novel *O*-PyBOP'ed product. The resin was subsequently treated with 1 M sodium methoxide in methanol to remove any O-modification.[20] Analytical cleavages revealed the expected products. Next, the scaffold was N-acylated with hexanoyl or heptanoyl chloride in CH_2Cl_2 in the presence of DIEA. As in the model studies, treatment with $SnCl_2 \cdot 2H_2O$ in DMF smoothly reduced the nitro group to an amino group.

In the second N-acylation the resin was treated with benzoyl chloride or phenylacetyl chloride in DCM in the presence of DIEA for 2 h, followed by treatment with 1 M sodium methoxide in MeOH to remove any O-acylation. In a separate procedure the resin was subjected to reductive amination by treatment with 5 equiv each of benzaldehyde and $NaBH_3CN$ in DMF−HOAc (99:1) for 10 h. Finally, the completed resins were treated with TFA−water (19:1) to release the crude products which were purified by silica gel chromatography giving isolated yields ranging from 10 to 40% over 6 steps.

Conclusions

Using the BAL handle we were able to develop a solid-phase strategy *not* requiring protecting groups for the synthesis of hapalosin mimetics. The synthesis strategy allowed for two chemoselective acylations followed by either further acylation or reductive aminations to yield hapalosin mimetics displaying four different structural domains designed to occupy similar spatial positions as in the parent natural product. A small 12-member combinatorial library

Scheme 5. LC and LC−HRMS Analysis of Crude Cleavage Product after the First Derivatization[a]



[a] The lipophilic peak at 5.45 min corresponds to the tentatively assigned *O*-PyBOP conjugated adduct.

Table 1. Structures of Hapalosin Mimetics

compd	R1	R2-C(O)	R3-C(O) or R3-CH_2
6Aa	isopropyl	hexanoyl	benzoyl
6Ab	isopropyl	hexanoyl	phenacetyl
6Ac	isopropyl	hexanoyl	benzyl
6Ba	isopropyl	heptanoyl	benzoyl
6Bb	isopropyl	heptanoyl	phenacetyl
6Bc	isopropyl	heptanoyl	benzyl
7Aa	isobutyl	hexanoyl	benzoyl
7Ab	isobutyl	hexanoyl	phenacetyl
7Ac	isobutyl	hexanoyl	benzyl
7Ba	isobutyl	heptanoyl	benzoyl
7Bb	isobutyl	heptanoyl	phenacetyl
7Bc	isobutyl	heptanoyl	benzyl

(Table 1) has been synthesized in good to excellent yields, and all 12 products had the expected analytical data (^{13}C or ^1H NMR and MS). This first generation library is currently undergoing screening.

Experimental Section

General Methods. $NaBH_3CN$ and DIEA were purchased from Sigma-Aldrich, PyBOP and aminomethyl polystyrene resin were from NovaBiochem. Dichloromethane (DCM) was distilled from CaH_2. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were distilled from Na/benzophenone. Dimethylformamide (DMF) and DMF−HOAc (99:1) were

dried over 3 Å molecular sieves. 1,2-Dichloroethane (DCE) was dried over 4 Å molecular sieves. Analytical grade solvents were used for washings of resins and for extractions. Evaporations were performed on a rotary evaporator at <40 °C. Flash chromatography was performed on silica (Merck 1.09385). HPLC was performed on a YMC FL-C8-3 (50 × 4.6, 3 μm) column using a Merck Hitachi LaChrom HPLC system equipped with a L-7100 pump and a L-7450 diode array detector. Buffer A was 0.05% TFA in H_2O, and buffer B was 0.035% TFA in CH_3CN. Gradient (0.0 min, 100% A; 0.5−8.0 min, 100% A−30% A) UV detection at 220 nm. ^{1}H NMR spectroscopy was performed on a Varian Unity Inova 500 operating at 499.87 MHz equipped with a z-(single axis) PFG inverse detection C−H−P probe. ^{13}C spectroscopy was performed on a Varian Unity Inova 500 operating at 125.6 MHz. High-resolution mass spectra (HRMS) were measured on a Micromass LCT. TLC was performed on aluminum plates (Merck 1.05554), and preparative TLC plates were made from Merck Silica 60PF_{254} on 20 × 40 cm glass plates in a 1 mm thickness. All solid-phase reactions where performed in polypropylene plastic syringes fitted with a polyethylene filter and equipped with a Teflon stopcock at room temperature on an horizontal shaker.

4-Hydroxy-3,5-dinitrobenzoic Acid, 3. 4-Chloro-3,5-dinitrobenzoic acid (12.0 g, 48.6 mmol) was added to a stirred solution of NaOH (6.8 g, 170.1 mmol) in H_2O (200 mL). After being stirred for 18 h, the solution was acidified with concentrated aqueous HCl (12 mL) and then filtered to give a yellow solid in 98% yield (10.4 g): mp 244−246 °C (lit.[11b] 248 °C).

3-Amino-4-hydroxy-5-nitrobenzoic Acid, 2. 4-Hydroxy-3,5-dinitrobenzoic acid (5.0 g, 21.9 mmol) was dissolved in MeOH (250 mL), and Pd/C (250 mg) was added under argon. The reaction mixture was hydrogenated at atmospheric pressure for 18 h until 1200 mL of hydrogen had been consumed. The reaction mixture was filtered through Celite and concentrated. The black solid was recrystallized from H_2O (200 mL), after treatment with charcoal, to give dark red needles in 58% yield (2.5 g): mp 236−238 °C (lit.[11b] 230−235 °C decomp.).

PALdehyde Resin. *o*-PALdehyde[16] (2.15 g, 7.50 mmol), PyBOP (4.16 g, 8.00 mmol), and DIEA (2.61 mL, 15.00 mmol) were disssolved in DMF (30 mL). Following 1 min of preactivation, the coupling mixture was added to amino-methyl polystyrene resin (1.0 mmol/g, 5.0 g) in DMF (20 mL). After 18 h and a negative Kaiser test, the resin was filtered and washed with DMF (6 × 35 mL). Upon capping with Ac_2O−DMF (1:20, 40 mL) for 30 min, the resin was filtered and washed with DMF (6 × 35 mL).

Loading of 3-Amino-4-hydroxy-5-nitrobenzoic Acid to PALdehyde Resin. PALdehyde PS resin (5.0 mmol) was washed twice with DMF−HOAc (99:1, 40 mL). Following addition of DMF−HOAc (99:1, 40 mL) and 3-amino-4-hydroxy-5-nitrobenzoic acid (1.98 g,10 mmol), $NaBH_3CN$ (3.14 g, 50 mmol) was added over 10 min. After 48 h the resin was filtered and washed with DMF (5 × 35 mL), MeOH (3 × 50 mL), and DCM (3 × 35 mL) and then dried overnight under high vacuum.

Determination of Loading Level. The loading level was determined by cleavage of resin (2 × 5.2 mg) with TFA−water (19:1, 0.5 mL) for 3 h. The resin was filtered off and washed twice with 0.5 mL of TFA. Upon evaporation of TFA the residue was dissolved in CH_3CN-H_2O (2:3, 1.00 mL) and analyzed by HPLC. From a standard curve of 3-amino-4-hydroxy-5-nitrobenzoic acid the loading level was determined to 0.65 mmol/g (loading efficiency of 94% based on the 1.00 mmol loading level for the aminomethyl polystyrene resin). No corrections were made for the loading level throughout the synthesis.

Model Studies. Optimization of Activation and Amidation with 2-Aminopropane (Step a). PALdehyde resin (1.2 mmol/g, 10 mg resin) was allowed to swell for 5 min in DMF, and then coupling reagent (HATU or PyBOP), auxiliary nucleophile (HOAt or HOBt), and DIEA were added as solutions in DMF. Following a preactivation period (0, 5, 10 min), 2-aminopropane was added as a solution in DMF to give a final volume of 125 μL. After 3 h the resin was filtered and washed with DMF (2 × 3 mL), DMF−HOAc (10:1, 2 × 3 mL), and DCM (4 × 3 mL). After treatment with TFA−water (19:1, 0.5 mL) for 2 h, the resin was filtered and washed with TFA−water (19:1, 2 × 0.5 mL). The combined TFA solutions where concentrated, redissolved in CH_3CN-H_2O (2:3, 5 mL), and analyzed by HPLC. Optimum conditions were PyBOP in the presence of DIEA *without* preactivation.

Optimization of First N-Acylation (Step b). 2-Aminopropyl amide resin (10 mg) was allowed to swell for 5 min in the chosen solvent (DCE, DCM, DMF, and pyridine). Hexanoyl chloride (5 or 10 equiv), HOAt or HOBt (0 or 5 equiv), or DMAP (none or catalytic amount) was added as a 25% solution in the chosen solvent to the resin, together with base (DIEA or 2,6-di-*tert*butyl-4-methylpyridine) also as a 25% solution, to a final volume of 125 μL. After the indicated time (2, 4, or 18 h), the resin was washed with DCM (5 × 3 mL) and MeOH (5 × 3 mL). One-half of the resin was cleaved with TFA−water (19:1, 0.5 mL) and analyzed by HPLC. The other half was treated with 5 equiv of $NaOCH_3$ in MeOH−THF (1:3). The resin was then washed with MeOH (1 × 3 mL), DMF (4 × 3 mL), DMF−HOAc (1 × 3 mL), MeOH (3 × 3 mL), and DCM (3 × 3 mL). After cleavage with TFA−water (19:1, 0.5 mL), the product was analyzed by HPLC. Optimum conditions were hexanoyl chloride in DCM in the sole presence of DIEA.

Optimization of Reduction of Nitro Group to Amine (Step c). A 2-aminopropyl amide and hexanoylated resin derivative (50 mg) was allowed swell in DMF (3 mL) for 5 min. The resin was washed once with 2 M $SnCl_2 \cdot 2H_2O$ in DMF before adding 2 M $SnCl_2 \cdot 2H_2O$ in DMF (3 mL). The resin changed color from red-orange to yellow during the reduction. After 30, 60, and 120 min, respectively, an aliquot of resin (∼10 mg) was removed for analysis and washed with DMF (5 × 3 mL), MeOH (3 × 3 mL), and DCM (3 × 3 mL), followed by treatment with TFA−water (19:1, 0.5 mL). The reaction was complete within 30 min.

Second Acylation (Step d). A 2-aminopropyl amide, hexanoylated, and reduced resin derivative (10 mg) was swollen in DCM (3 mL) for 5 min. Benzoyl chloride (37

μmol, 5 equiv) and DIEA (5 equiv) were added, respectively, as a 25% solution in DCM to a final volume of 125 μL. After 2 h, the resin was washed with DMF (5 × 3 mL), MeOH (3 × 3 mL), and DCM (3 × 3 mL). The acylation was repeated once to ensure full conversion. Finally, treatment with TFA−water (19:1, 0.5 mL) released the crude product which was analyzed by HPLC.

Optimization of Reductive Amination (Step e). A 2-aminopropyl amide, hexanoylated, and reduced resin derivative (10 mg) was allowed to swell for 5 min in HOAc−DMF (1:99) or HOAc−THF (1:99), respectively. Benzaldehyde (1.5, 2, 2.5, 3, or 5 equiv) and $NaBH_3CN$ (3 equiv or 5 equiv) were added as 25% solutions in HOAc−DMF or HOAc−THF to a final volume of 125 μL. After (1, 2, 4, 8, 10, and 18 h), the resin was washed with DMF (5×), MeOH (3×), and DCM (3×). Treatment with TFA−water (19:1, 0.5 mL) released the crude product which was analyzed by HPLC. Optimum conditions were benzaldehyde (5 equiv) and $NaBH_3CN$ (5 equiv) in DMF−HOAc (99:1) for 8 h; HPLC analysis showed quantitative consumption of the starting material and 88% of the major product and 12% of byproduct.

Library Synthesis. General Procedure for Amidation (Step a). DMF (35 mL), DIEA (2.35 mL, 13.50 mmol), and amine (2-aminopropane or 2-methyl-1-aminopropane, 22.5 mmol) were added to resin-bound 3-amino-4-hydroxy-5-nitrobenzoic acid (6.92 g, 4.50 mmol). A solution of PyBOP (7.02 g, 13.5 mmol) in DMF (10 mL) was added over 5 min to the resin. After 3 h, the resin was filtrated, washed with DMF (5 × 35 mL), MeOH (3 × 45 mL), THF (39 mL), and MeOH (4 mL), and treated with 1 M $NaOCH_3$ in MeOH (9.00 mL, 9.00 mmol) for 30 min. The resin was filtered and washed with DMF (4 × 40 mL), DMF:AcOH (10:1, 1 × 40 mL), MeOH (3 × 40 mL), and DCM (3 × 40 mL). The following analytical cleavages were carried out with TFA−H_2O (19:1) on 5−10 mg of resin.

3-Amino-4-hydroxy-5-nitro-*N*-isopropylbenzamide, 5. A portion of the resin was cleaved and analyzed. Calcd for $C_{10}H_{13}O_4N_3$ 239.0906, LC−MS: 240.0977 [MH^+].

General Procedure for Acylation (Step b). To resin-bound 3-amino-4-hydroxy-*N*-isopropyl-5-nitrobenzamide (3.38 g, 2.20 mmol) were added DCM (30 mL), DIEA (1.92 mL, 11.00 mmol), and acid chloride (hexanoyl chloride or heptanoyl chloride (11 mmol). After 2 h, the resin was washed with DCM (1 × 50 mL), DMF (1 × 50 mL), and MeOH (3 × 50 mL). THF (30 mL), MeOH (5.6 mL), and 1 M $NaOCH_3$ in MeOH (4.40 mL, 4.40 mmol) were added. After 30 min, the resin was filtered and washed with MeOH (1 × 30 mL), DMF (4 × 30 mL), DMF−HOAc (10:1, 1 × 30 mL), MeOH (3 × 30 mL), and DCM (3 × 30 mL). The resin was dried overnight under high vacuum.

3-(Hexanoylamino)-4-hydroxy-5-nitro-*N*-isopropylbenzamide. A small portion of the resin was cleaved and analyzed. Calcd for $C_{17}H_{23}O_5N_3$ 337.1638, LC−MS: 338 [MH^+].

General Procedure for $SnCl_2$ Reduction (Step c). The resin (3.07 g, 2.00 mmol) was washed with DMF (30 mL) and then treated with freshly prepared 2 M $SnCl_2 \cdot 2H_2O$ in DMF (30 mL). After 40 min the resin was filtered, washed with DMF (5 × 30 mL), MeOH (3 × 30 mL), and DCM (3 × 30 mL), and dried overnight under high vacuum.

3-Amino-5-(hexanoylamino)-4-hydroxy-*N*-isopropylbenzamide. A small portion of the resin was cleaved and analyzed. Calcd for $C_{17}H_{25}O_3N_3$ 307.1896, LC−MS: 308 [MH^+].

General Procedure for the Second Acylation (Step d). To the anilino resin (385 mg, 0.25 mmol) were added DCM (4 mL), DIEA (0.218 mL, 1.25 mmol), and acid chloride (benzoyl chloride or phenylacetyl chloride; 0.164 mL, 1.25 mmol). After 2 h the resin was washed with DCM (2 × 10 mL), and the acylation procedure was repeated. The resin was filtered and washed with DCM (5 × 10 mL), DMF (5 × 10 mL), and MeOH (5 × 10 mL). The resin was dried overnight under high vacuum. Treatment with TFA−water (19:1, 4 mL) for 2 h released the products into solution. The resin was filtered off and rinsed twice with TFA (2 mL). The combined TFA solutions were concentrated and re-evaporated from diethyl ether (2×), DCE (2×), and diethyl ether (2×). The products were dried under high vacuum, and the crude yield was determined. The products were purified by flash chromatography or preparative TLC.

General Procedure for Reductive Amination (Step e). DMF−HOAc (99:1, 1.53 mL) and benzaldehyde (0.127 mL, 1.25 mmol) were added to anilino-resin (385 mg, 0.25 mmol) together with a solution of $NaBH_3CN$ (78.6 mg, 1.25 mmol) in DMF−HOAc (99:1, 0.77 mL). After 10 h the resin was filtered and washed with DMF (5 × 10 mL), MeOH (3 × 10 mL), and DCM (3 × 10 mL). The resin was dried overnight under high vacuum.

General Procedure for Release from Solid Support (Step f). The completed resins were treated with TFA−water (19:1, 4 mL) for 2 h. The resin was filtered and rinsed twice with TFA (2 mL). After concentration of the combined TFA solutions, EtOAc (10 mL) was added and the organic phase extracted with brine (10 mL) and saturated $NaHCO_3$ (0.5 mL). The aqueous phase was extracted with EtOAc (2 × 10 mL), and the combined organic phases were dried over Na_2SO_4 and concentrated. The crude products were dried under high vacuum overnight, and the crude yield was determined. Products were purified by flash chromatography or preparative TLC.

3-Benzoylamino-5-hexanoylamino-4-hydroxy-*N*-isopropylbenzamide (6Aa). After cleavage, a crude yield of 88 mg (86%) of brownish oil was obtained with a purity of 87% by rp-HPLC. Preparative TLC (DCM−MeOH, 24:1) afforded 35.4 mg (34%) of a light yellow solid: ^{13}C NMR ($CDCl_3$) δ 174.5, 166.7, 166.5, 141.3, 134.1, 132.2, 128.8, 128.7, 127.4, 126.0, 116.8, 114.9, 42.1, 36.8, 31.3, 25.4, 22.7, 22.4, 13.9. HRMS ($C_{23}H_{30}N_3O_4$) calcd [M + H]$^+$, 412.2236; found, 412.2266.

3-Hexanoylamino-4-hydroxy-5-(2-phenylacetyl)-*N*-isopropylbenzamide (6Ab). After cleavage, a crude yield of 101 mg (95%) of brownish oil was obtained with a purity of 86% by rp-HPLC. Preparative TLC (DCM−MeOH, 24:1) afforded 10.1 mg (10%) of light yellow solid: ^1H NMR ($CDCl_3$) δ 10.94 (s, 1H), 9.07 (s, 1H), 8.62 (s, 1H), 8.15 (s, 1H), 7.97 (s, 1H), 7.45−7.30 (m, 5H), 6.21 (d, 1H), 4.20 (m, 1H), 3.81 (s, 2H), 2.48 (6, J = 7.7 Hz, 2H), 1.72 (t, J =

7.5 Hz, 2H), 1.37−0.84 (m, 11H). HRMS ($C_{24}H_{32}N_3O_4$) calcd $[M + H]^+$, 426.2393; found, 426.2444.

3-Benzylamino-5-hexanoylamino-4-hydroxy-*N*-isopropylbenzamide (6Ac). After cleavage and extraction, a crude yield of 55 mg (55%) of yellow solid was obtained with a purity of 77% by rp-HPLC. Flash chromatography (hexanes−EtOAc, 3:2) afforded 27.0 mg (27%) of light yellow crystals: mp 165−167 °C; ^{13}C NMR ($CDCl_3$) δ 174.7, 167.3, 139.4, 138.7, 128.7, 127.6, 127.4, 126.7, 125.2, 48.5, 41.9, 36.5, 31.3, 25.5, 22.8, 22.4, 13.9. HRMS ($C_{23}H_{32}N_3O_3$) calcd $[M + H]^+$, 398.2444; found, 398.2440.

3-Benzoylamino-5-heptanoylamino-4-hydroxy-*N*-isopropylbenzamide (6Ba). After cleavage, a crude yield of 93 mg (87%) of brownish oil was obtained with a purity of 88% by rp-HPLC. Flash chromatography (hexanes−EtOAc, 1:2) afforded 42.3 mg (40%) of light yellow solid: ^{13}C NMR ($CDCl_3$) δ 174.5, 166.7, 166.6, 141.6, 133.7, 132.3, 128.8, 128.3, 127.4, 127.1, 125.0, 117.0, 115.2, 42.4, 36.8, 31.5, 28.8, 25.6, 22.4, 22.4, 14.0. HRMS ($C_{24}H_{32}N_3O_4$) calcd $[M + H]^+$, 426.2393; found, 426.2436.

3-Heptanoylamino-4-hydroxy-5-(2-phenylacetyl)-*N*-isopropylbenzamide (6Bb). After cleavage, a crude yield of 101 mg (92%) of brownish oil was obtained with a purity of 77% by rp-HPLC. Flash chromatography (DCM−MeOH, 100:0→95:5) afforded 24.4 mg (22%) of light yellow solid: ^{13}C NMR ($CDCl_3$) δ 174.2, 170.9, 166.6, 146.7, 141.0, 134.0, 129.4, 129.1, 128.1, 127.6, 127.4, 125.6, 116.4, 114.8, 44.3, 42.2, 36.9, 31.5, 28.8, 25.6, 22.7, 22.4, 14.0. HRMS ($C_{25}H_{34}N_3O_4$) calcd $[M + H]^+$, 440.2549; found, 412.2583.

3-Benzylamino-5-heptanoylamino-4-hydroxy-*N*-isopropylbenzamide (6Bc). After cleavage and extraction, a crude yield of 48 mg (47%) of yellow solid was obtained with a purity of 77% by rp-HPLC. Flash chromatography (hexanes−EtOAc, 3:2) afforded 22.9 mg (22%) of light yellow crystals: mp 143−146 °C; ^{13}C NMR ($CDCl_3$) δ 174.7, 167.4, 146.7, 139.3, 128.6, 128.5, 127.5, 127.3, 126.7, 125.1, 111.3, 104.7, 48.3, 41.9, 36.5, 31.5, 28.8, 25.8, 22.8, 22.5, 20.1, 14.0. HRMS ($C_{24}H_{34}N_3O_3$) calcd $[M + H]^+$, 412.2600; found, 412.2635.

3-Benzoylamino-5-hexanoylamino-4-hydroxy-*N*-isobutylbenzamide (7Aa). After cleavage, a crude yield of 102 mg (96%) of brownish oil was obtained with a purity of 94% by rp-HPLC. Flash chromatography (hexanes−EtOAc−HOAc 22:3:1→15:9:1) afforded 33.8 mg (32%) of a light yellow solid: ^{13}C NMR ($CDCl_3$) δ 174.5, 167.6, 166.7, 146.7, 141.4, 134.1, 132.2, 128.8, 128.7, 127.4, 125.8, 116.8, 115.0, 47.6, 36.8, 31.3, 28.5, 25.4, 22.4, 20.2, 13.9.

3-Hexanoylamino-4-hydroxy-5-(2-phenylacetyl)-*N*-isobutylbenzamide (7Ab). After cleavage, a crude yield of 102 mg (96%) of brownish oil was obtained with a purity of 86% by rp-HPLC. Flash chromatography (DCM−MeOH, 100:0→98:2) afforded 28.2 mg (26%) of light yellow amorphous solid: ^{13}C NMR ($CDCl_3$) δ 174.2, 170.9, 167.6, 140.9, 134.0, 129.4, 129.1, 128.2, 127.6, 127.5, 125.9, 116.4, 114.7, 47.7, 44.4, 36.9, 31.3, 28.5, 25.3, 22.4, 20.2, 13.9. HRMS ($C_{25}H_{34}N_3O_4$) calcd $[M + H]^+$, 440.2549; found, 440.2549.

3-Benzylamino-5-hexanoylamino-4-hydroxy-*N*-isobutylbenzamide (7Ac). After cleavage and extraction, a crude yield of 62 mg (61%) of yellow solid was obtained with a purity of 82% by rp-HPLC. Flash chromatography (hexanes−EtOAc, 2:1) afforded 34.4 mg (33%) of light yellow crystals: mp 107−109 °C; ^{13}C NMR ($CDCl_3$) δ 174.7, 168.3, 139.9, 139.3, 138.9, 128.6, 127.3, 127.2, 126.6, 125.1, 111.3, 104.6, 48.2, 47.4, 36.4, 31.3, 28.4, 25.5, 22.4, 20.1, 13.9. HRMS ($C_{24}H_{34}N_3O_3$) calcd $[M + H]^+$, 412.2600; found, 412.2600.

3-Benzylamino-5-heptanoylamino-4-hydroxy-*N*-isobutylbenzamide (7Ba). After cleavage, a crude yield of 110 mg (100%) of brownish oil was obtained with a purity of 91% by rp-HPLC. Preparative TLC (DCM−MeOH, 97:3) afforded 33.9 mg (31%) of light yellow amorphous solid: ^{13}C NMR ($CDCl_3$) δ 174.5, 167.7, 166.7, 141.5, 134.0, 132.2, 128.8, 128.6, 127.4, 127.4, 125.7, 116.8, 115.0, 47.7, 36.9, 31.5, 28.9, 28.5, 25.7, 22.5, 20.2, 14.0. HRMS ($C_{25}H_{34}N_3O_4$) calcd $[M + H]^+$, 440.2549; found, 440.2581.

3-Heptanoylamino-4-hydroxy-5-(2-phenylacetyl)-*N*-isobutylbenzamide (7Bb). After cleavage, a crude yield of 120 mg (106%) of brownish oil was obtained with a purity of 83% by rp-HPLC. Preparative TLC (DCM−MeOH, 97:3) afforded 32.5 mg (29%) of light yellow solid: ^{13}C NMR ($CDCl_3$) δ 174.2, 170.9, 167.5, 141.0, 134.0, 129.4, 129.1, 128.2, 127.6, 127.4, 125.7, 116.4, 114.8, 47.6, 44.3, 36.9, 31.5, 28.8, 28.5, 25.6, 22.4, 20.1, 14.0. HRMS ($C_{26}H_{36}N_3O_4$) calcd $[M + H]^+$, 454.2706; found, 454.2737.

3-Benzylamino-5-heptanoylamino-4-hydroxy-*N*-isobutylbenzamide (7Bc). After cleavage and extraction, a crude yield of 70 mg (66%) of yellow solid was obtained with a purity of 81% by rp-HPLC. Column chromatography (hexanes−EtOAc, 2:1) afforded 24.5 mg (23%) of light yellow crystals: mp 99−102 °C; ^{13}C NMR ($CDCl_3$) δ 174.7, 168.3, 139.4, 138.7, 128.6, 127.4, 127.3, 126.6, 125.2, 111.5, 48.3, 47.4, 36.5, 31.5, 28.8, 28.5, 25.8, 22.5, 20.1, 14.0. HRMS ($C_{25}H_{36}N_3O_3$) calcd $[M + H]^+$, 426.2757; found, 426.2792.

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