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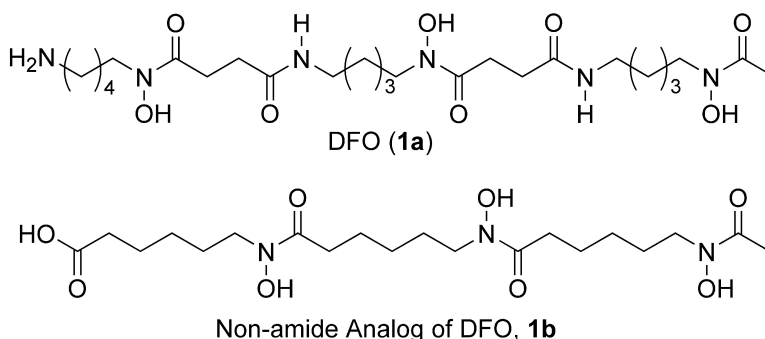
Hydroxamate-Based Iron Chelators: Combinatorial Syntheses of Desferrioxamine B Analogues and Evaluation of Binding Affinities

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DFO (**1a**)

Non-amide Analog of DFO, **1b**

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Hydroxamate-Based Iron Chelators: Combinatorial Syntheses of Desferrioxamine B Analogues and Evaluation of Binding Affinities

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This article describes the solid-phase combinatorial methods developed for the synthesis of polyhydroxamate-based siderophores. This strategy was applied to generate several libraries of structural DFO (**1a**) analogues that include DFO variants, non-amide analogues, C-terminal modified analogues, reverse-amide analogues, and hybrid analogues. To assess the relative iron-binding affinities of these compounds, a high-throughput spectrophotometric screening method based on competition with 8-hydroxyquinoline-5-sulfonic acid was developed. Some of the promising candidates containing various terminal functional groups were identified and prepared on large scale to enable future studies in animal models for iron-overload diseases.

Introduction

Desferrioxamine B (Desferal, DFO, **1a**), a siderophore derived from *Streptomyces pilosis*,[1] contains three hydroxamate groups that form very stable hexadentate complexes with ferric iron ($K_f = 1 \times 10^{30}$ M^{-1}).[2] This chemical characteristic has made DFO a routinely used treatment over the last 30 years for iron overload caused by the frequent blood transfusions during the treatment of thalassemias and sickle cell anemia.[3,4] Clinical experience has shown that infusion of DFO, which is not orally active, results in problems with noncompliance and toxic side effects.[5,6] Noncompliance is attributed to the necessity for continuous daily infusion therapy. Since transfusional iron overload is the most common condition of metal toxicity worldwide as well as having the highest mortality rate,[7] a less toxic, oral replacement for the treatment of transfusional iron overload is long overdue.[8] The need for an oral treatment was realized somewhat when Ferriprox (deferiprone, L_1; marketed by Apotex, Inc., Toronto, Canada), a bidentate ligand, was approved as a second-line therapy in Europe for those patients unable to tolerate Desferal, but some concerns over long-term toxicity linger.[9] Many of these toxic side effects can be attributed to the lack of selectivity of this drug for ferric iron. In addition, long-term studies also question the efficacy of deferiprone in contrast to preliminary results.[10] Thus, the successful development of an orally active, nontoxic, selective iron chelator is still highly desirable.

Our approach to developing orally active iron chelators has been to synthesize focused libraries of DFO analogues that allowed exploration of lipophilicity, chemical groups, and molecular weight on the biodistribution and removal of iron. The solution-phase syntheses of several important siderophores, including DFO and analogues,[11−16] have been accomplished. For purposes of developing a structure−activity relationship of DFO, the synthesis of individual libraries in solution would be a very daunting, time-consuming task. Synthesizing libraries using solid-phase combinatorial chemistry allows the efficient synthesis of a myriad of structurally diverse DFO analogues without time-consuming purification and characterization of intermediates. The focus of this report is the use of DFO as a starting point to introduce modifications into its backbone in order to address its clinical inadequacies.

Our strategy involved the repetitive assembly of the hydroxamate unit on a solid support through the intermediacy of an orthogonally N,O-bisprotected hydroxylamine. The hydroxylamine nitrogen was protected with the 2-nitrophenylsulfonyl (nosyl) group, thus allowing activation of the nitrogen for functionalization via alkylation with alkyl halides as well as coupling with alcohols under Mitsunobu conditions (Scheme 1). The choice of the nosyl group, first introduced by Fukuyama et al.[17] in the N-alkylation of the primary amines, was based on its reported utility in the generation of *N*-alkyl peptides on the solid support.[18−20] The selective removal of the nosyl group with thiolate anion facilitates another site for functionalization through N-acylation. The hydroxyl group was protected with an acid-sensitive protecting group, allowing simultaneous deprotection and cleavage from the solid support. The multigram synthesis of *N*-nosyl-*O*-protected hydroxylamines and the utility of the *O-tert*-butyl analogue in the solution synthesis of a trihydroxamate **1b** (Figure 1) has been described previously.[21]

We selected a Wang matrix as the basic solid-phase support due its stability under a wide range of conditions and the ease with which the synthesized material may be cleaved off the polymer support. The initial solid-phase

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Scheme 1. Synthesis of Hydroxamate Unit on Solid-Phase
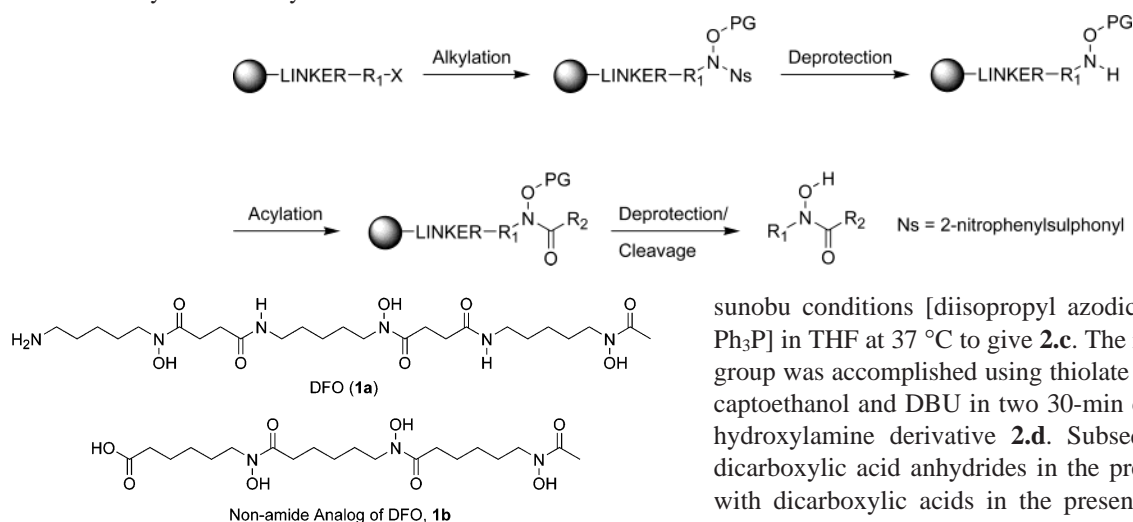
Figure 1. Structures of DFO and a non-amide analogue of DFO.

synthesis of DFO (**1a**) and a non-amide analogue **1b** employed benzyl protection for the hydroxyl group in order to facilitate optimization and characterization at each synthetic step by UV absorption. This necessitated catalytic hydrogenation following cleavage of the target molecule from the resin to remove benzyl groups. To make high-throughput synthesis more feasible, the hydroxyl-protecting group was changed to *tert*-butyl during the synthesis of **1a** and **1b**, to enable deprotection and cleavage from the resin in a single step using TFA. The shift in protecting group required that each step be completely reoptimized, due to differences in reactivity of the substrates protected by the *tert*-butyl group. Our goals at this stage were to maximize yield and purity in each step, reduce the overall time of the reaction sequence to speed library production, and ensure compatibility of reactants at each step with robotic liquid-handling systems used by automated synthesizers (manuscript in preparation).
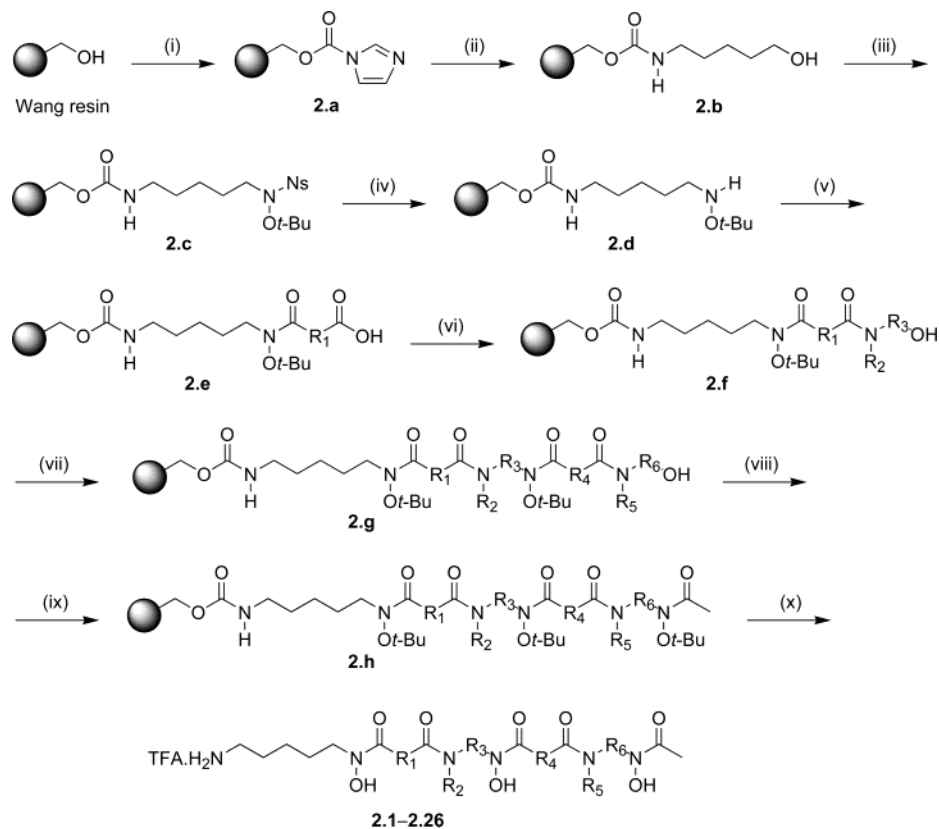
This paper describes several prototype libraries made by parallel synthesis and explores the range of reactants compatible with the optimized synthetic strategy. "Variants" refers to compounds made by simple substitution of reagents within the same reaction sequence. "Analogues" refers to any compounds in which the DFO backbones have been modified and, consequently, require a modified reaction sequence. All of the library compounds were characterized by RP-HPLC and MS (ESI) analyses. To assess the impact of structural variation on ferric ion affinity, a high-throughput screening assay was developed, the details of which are also discussed. Some of these results were presented at various meetings,[22,23] and an overview has appeared in a review.[24]

Results and Discussion

DFO Variants. An initial library of 92 DFO variants, with terminal amino groups similar to that in DFO itself, was planned and executed as described in Scheme 2. The first step involved activation of Wang resin as the imidazolide carbamate using 1,1′-carbonyldiimidazole (CDI),[25] followed by reaction with 5-amino-1-pentanol at 60 °C in DMF. The resulting resin-bound alcohol **2.b** was reacted with *O-tert*-butyl-*N*-nosylhydroxylamine (*t*-BuO-NH-Ns) under Mit-

sunobu conditions [diisopropyl azodicarboxylate (DIAD), Ph₃P] in THF at 37 °C to give **2.c**. The removal of the nosyl group was accomplished using thiolate generated by 2-mercaptoethanol and DBU in two 30-min cycles to furnish the hydroxylamine derivative **2.d**. Subsequent reaction with dicarboxylic acid anhydrides in the presence of DMAP or with dicarboxylic acids in the presence of *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) and *N,N*-diisopropylethylamine (DIPEA) in dimethylacetamide (DMA) afforded the acid derivative **2.e**. The synthesis of the remainder of the sequence involved the repetition of the appropriate synthetic steps with required diversity reagents, followed by reaction with acetic anhydride and pyridine in DMF, ultimately leading to **2.h**. After the resin-bound products were dried, they were cleaved from the solid support using dichloromethane (DCM/TFA (1:9), and the solutions in screw-capped vials were left overnight at room temperature to effect the deprotecton of the *tert*-butyl groups. After removal of the solvent, the residues were triturated with acetonitrile, to obtain **2.1−2.26** as solids.

With various dicarboxylic acids (succinic anhydride, glutaric anhydride, 3,3-tetramethyleneglutaric anhydride, adipic acid, 1,4-phenylenedipropionic acid, *trans*-1,4-cyclohexanedicarboxylic acid, and 3,6,9-trioxaundecanedioic acid) and amino alcohols [5-amino-1-pentanol, 4-piperidineethanol, L-alaninol, (*S*)-leucinol, 3-amino-1-propanol, and 4-aminophenethyl alcohol], 26 DFO variants, **2.1−2.26** were obtained (Table 1). It was found that acid anhydrides work best in the coupling. When anhydrides could not be purchased, the corresponding diacid was activated with HATU in order to obtain reasonable yields. Unfortunately, in the subsequent step, the high reactivity of the activated ester resulted in reduced chemoselectivity for the amino group of the amino alcohol in some cases, particularly for aromatic amines. 3,6,9-Trioxaundecanedioic acid gave mixtures, which may arise during treatment of TFA and reflect the instability of the ether under these conditions. In this case, a THP or a 2,4-dimethoxybenzyl (DMB) protection strategy should be preferred over *tert*-butyl, in which less drastic deprotection conditions are required in the final step. Under subsequent Mitsunobu reaction conditions, we suspect that alaninol and leucinol cyclize to form aziridine or oxazoline structures.[26] Dehydration of the alcohol may also be involved, in particular, when it leads to a conjugated system. Some of these issues underline the complex nature of several steps of this library, and only 28% of attempted compounds were obtained with reasonable RP-HPLC purity and MS (ESI) data.

Scheme 2. Solid-Phase Synthesis of DFO Variants[a]

Wang resin — (i) → 2.a — (ii) → 2.b — (iii) →

2.c — (iv) → 2.d — (v) →

2.e — (vi) → 2.f

(vii) → 2.g — (viii) →

(ix) → 2.h — (x) →

2.1–2.26

[a] Reagents and conditions: (i) CDI, THF−DMF (4:1), 2 h; (ii) H$_2$N(CH$_2$)$_5$OH, DIPEA, DMF, 60 °C, 24 h; (iii) t-BuO−NH−Ns, Ph$_3$P, DIAD, THF, 37 °C, 4 h; (iv) HS(CH$_2$)$_2$OH, DBU, DMF, 30 min (× 2); (v) dicarboxylic acid anhydride, DMAP, DMA or dicarboxylic acid, HATU, DIPEA, DMA, 50 °C, 8 h; (vi) repeat step (i); amino-alcohol, DIPEA, DMA, 12 h; (vii) repeat steps (iii) through (vi); (viii) repeat steps (iii) and (iv); (ix) Ac$_2$O, pyridine, DMF, 6 h; (x) TFA−DCM (9:1), 30 min, filter, and then 22 h.

Nonamide DFO Analogues. One major obstacle with DFO is that it has a very short half-life (5−10 min) in humans. The amide bonds in the DFO backbone are susceptible to proteolytic cleavage that facilitates the rapid clearance of DFO. One obvious approach to enhance the lipophilicity and increase the metabolic stability of DFO was to eliminate one or more amide groups. As a result, a combinatorial approach to prepare such analogues was developed (Scheme 3), in which the three hydroxamates needed for iron affinity were retained. Syntheses were carried out on Mimotopes pins with Wang-type resin having acid-labile phenoxyacetic acid linkers. Initial loading of the bromocarboxylic acid was carried out with 1,3-diisopropyl-carbodiimide (DIC) in THF in the presence of catalytic DMAP. The resulting resin-bound bromide **3.a** was heated with t-BuO-NH-Ns and DBU in DMF at 50 °C to give **3.b**. Subsequent removal of the nosyl group, followed by coupling with a bromocarboxylic acid in the presence of HATU and DIPEA furnished **3.d**. The remainder of the synthetic sequence is a reiteration of the appropriate steps (bromide displacement, denosylation, and acylation) before capping with an acetyl group and cleaving the products (**3.1**−**3.12**) from the solid support using TFA−DCM (9:1).

Out of the 27 possible compounds incorporating 4-bromobutyric-, 6-bromohexanoic-, and 8-bromooctanoic acids, 12 compounds were obtained with correct molecular ion peaks (Table 2). The butyryl residue, when not anchored to the resin and used in other positions, cyclized after the

deprotection of the nosyl group and prematurely terminated the chain. The other reagents worked well in all positions. A minor side reaction observed during synthesis involved displacement of bromide from the acid reagent by 7-aza-1-hydroxybenzotriazole (HOAt) during coupling with HATU, resulting in termination of the chain by ether formation. To circumvent this problem, in all subsequent syntheses, hydroxylamines of the type **3.c** were acylated with acid chlorides in the presence of DIPEA, rather than HATU-mediated coupling of the acids.

C-Terminal Modified Nonamide DFO Analogues. In a somewhat different approach, an additional N-hydroxylamino moiety was incorporated at the beginning of the hydroxamate chain (Scheme 4). The key step needed for this strategy was a method for linking the hydroxylamine group directly to the resin and using it as a starting point from which to build up the rest of the ligand. Methods in which the hydroxamate group was attached to either Sasrin,[27] trityl,[28−30] or Wang[31,32] resins through the hydroxyl group have been published. We have developed an alternate method that allows more diversity. By starting the sequence with hydroxylamine alkylation, a new venue is available by which to modify the lipophilicity of the compound. Hydroxylamine **4.a** was prepared from Wang resin by a reported procedure[31] via Mitsunobu conditions using N-hydroxyphthalimide, followed by removal of the phthaloyl group by hydrazinolysis. The nosyl group was introduced into **4.a** using 2-nosyl chloride and 2,6-lutidine as base under conditions that ensured

Table 1. RP-HPLC Purity and Masses Found for DFO Variants **2**[a]

product	R_1	NR_2R_3	R_4	NR_5R_6	MW calcd	MW found	purity[b] (%)
2.1[c]	$(CH_2)_2$	$NH(CH_2)_5$	$(CH_2)_2$	$NH(CH_2)_5$	560	561 $(M + H)^+$	67
2.2	$(CH_2)_2$	$NH(CH_2)_5$	$(CH_2)_3$	$NH(CH_2)_5$	574	575 $(M + H)^+$	63
2.3	$(CH_2)_2$	$NH(CH_2)_5$	$(CH_2)_3$	(A)	600	601 $(M + H)^+$	53
2.4	$(CH_2)_2$	$NH(CH_2)_5$	(B)	$NH(CH_2)_5$	664	665 $(M + H)^+$	37
2.5	$(CH_2)_3$	$NH(CH_2)_5$	$(CH_2)_2$	$NH(CH_2)_5$	574	575 $(M + H)^+$	53
2.6	$(CH_2)_3$	$NH(CH_2)_5$	$(CH_2)_3$	$NH(CH_2)_5$	588	589 $(M + H)^+$	67
2.7	$(CH_2)_3$	$NH(CH_2)_5$	$(CH_2)_3$	(A)	614	615 $(M + H)^+$	52
2.8	$(CH_2)_3$	$NH(CH_2)_5$	(B)	(A)	704	705 $(M + H)^+$	51
2.9	$(CH_2)_3$	(A)	$(CH_2)_2$	$NH(CH_2)_5$	600	601 $(M + H)^+$	59
2.10	$(CH_2)_3$	(A)	$(CH_2)_3$	$NH(CH_2)_5$	614	615 $(M + H)^+$	57
2.11	$(CH_2)_3$	(A)	$(CH_2)_3$	(A)	640	641 $(M + H)^+$	47
2.12	$(CH_2)_3$	(A)	(B)	$NH(CH_2)_5$	704	705 $(M + H)^+$	44
2.13	$(CH_2)_3$	(A)	(B)	(A)	730	731 $(M + H)^+$	38
2.14	$(CH_2)_4$	$NH(CH_2)_3$	$(CH_2)_2$	$NH(CH_2)_5$	560	561 $(M + H)^+$	39
2.15	(B)	$NH(CH_2)_5$	$(CH_2)_2$	$NH(CH_2)_5$	664	665 $(M + H)^+$	37
2.16	(B)	$NH(CH_2)_5$	$(CH_2)_3$	$NH(CH_2)_5$	678	679 $(M + H)^+$	37
2.17	(B)	$NH(CH_2)_5$	$(CH_2)_3$	(A)	704	705 $(M + H)^+$	36
2.18	(B)	$NH(CH_2)_5$	(B)	$NH(CH_2)_5$	766	767 $(M + H)^+$	31
2.19	(B)	$NH(CH_2)_5$	(B)	(A)	794	795 $(M + H)^+$	32
2.20	(B)	(A)	$(CH_2)_2$	$NH(CH_2)_5$	690	691 $(M + H)^+$	32
2.21	(B)	(A)	$(CH_2)_3$	$NH(CH_2)_5$	704	705 $(M + H)^+$	25
2.22	(B)	(A)	$(CH_2)_3$	(A)	730	731 $(M + H)^+$	46
2.23	(B)	(A)	(B)	$NH(CH_2)_5$	794	795 $(M + H)^+$	26
2.24	(B)	(A)	(B)	(A)	820	821 $(M + H)^+$	53
2.25	(C)	$NH(CH_2)_3$	$(CH_2)_2$	$NH(CH_2)_5$	600	601 $(M + H)^+$	25
2.26	(D)	$NH(CH_2)_3$	$(CH_2)_2$	$NH(CH_2)_5$	586	587 $(M + H)^+$	36

(A) = N—$(CH_2)_2$-   (B) = -$(CH_2)_2$—$(CH_2)_2$-   (C) =   (D) =
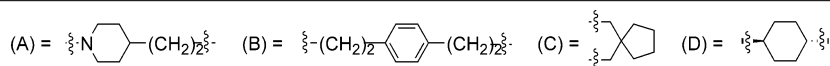
[a] The crude mass yields (as TFA salts) were in the range 49−114% with respect to the initial loading of the resin (1.1 mmol/g). [b] Purity was determined from the relative peak areas (%) of HPLC chromatograms (0−90% B/10 min). [c] DFO (**1a**).

Scheme 3. Solid-Phase Synthesis of Non-Amide DFO Analogues[a]
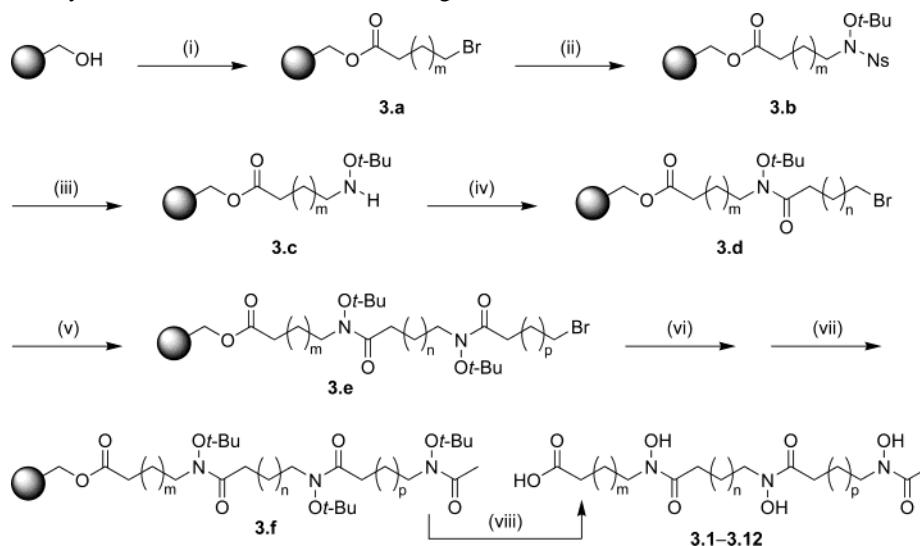
[a] Reagents and conditions: (i) bromocarboxylic acid, DIC, DMAP, THF, 1 h (× 2); (ii) t-BuO−NH−Ns, DBU, DMA, 50 °C, 2 h; (iii) HS$(CH_2)_2$OH, DBU, DMF, 30 min (× 2); (iv) bromocarboxylic acid, HATU, DIPEA, DMA, 4 h; (v) repeat steps (ii) through (iv); (vi) repeat steps (ii) and (iii); (vii) HOAc, HATU, DIPEA, DMA, 4 h; (viii) TFA−DCM (9:1), 3 h.

formation of the mononosyl derivative **4.b**. Subsequent reaction with alcohols under Mitsunobu conditions afforded the intermediate **4.c**. After nosyl group removal, the intermediate **4.d** was acylated with bromocarboxylic acid chloride in DCE in the presence of DIPEA. The resulting resin-bound bromide **4.e** was heated with t-BuO-NH-Ns and 1,1,3,3-tetramethylguanidine (TMG) in DMF at 50 °C to give **4.f**. The remainder of the synthetic sequence is reiterative (i.e.,

denosylation, acylation, and bromide displacement) before terminating with an acetyl group, and cleaving off the products (**4.1**−**4.27**) from the resin using TFA-triisopropyl-silane (TIS)−DCM (18:1:1) and leaving overnight at room temperature to effect the deprotection of the *tert*-butyl groups.

A prototype library of 24 compounds was prepared using MeOH, EtOH, BnOH, and *N*-(Boc)-5-amino-1-pentanol for alcohols and 6-bromohexanoyl and 8-bromooctanoyl chlo-

Table 2. RP-HPLC Purity and Masses Found for
Non-Amide DFO Analogues **3**[a]

product	m	n	p	MW calcd	MW found[b]	purity[c](%)
3.1	1	3	3	419	420 (M + H)$^+$	68
3.2	1	3	5	447	448 (M + H)$^+$	73
3.3	1	5	3	447	448 (M + H)$^+$	59
3.4	1	5	5	475	476 (M + H)$^+$	58
3.5[d]	3	3	3	447	448 (M + H)$^+$	61
3.6	3	3	5	475	476 (M + H)$^+$	78
3.7	3	5	3	475	476 (M + H)$^+$	78
3.8	3	5	5	503	504 (M + H)$^+$	78
3.9	5	3	3	475	476 (M + H)$^+$	63
3.10	5	3	5	503	504 (M + H)$^+$	63
3.11	5	5	3	503	504 (M + H)$^+$	63
3.12	5	5	5	531	532 (M + H)$^+$	67

[a] Carried out on the SynPhase Crowns (SP-PS-O-HMP, 2.2 μmol). [b] In MS (ESI$^-$), the molecular ions corresponded to (M $-$ H)$^-$. [c] Purity was determined from the relative peak areas (%) of HPLC chromatograms (10$-$90% B/9 min). [d] Non-amide DFO analogue **1b**.

rides for bromocarboxylic acid chlorides (Table 3). Compounds **4.1**, **4.8**, and **4.21** were prepared individually in polypropylene tubes prior to library synthesis while optimizing the chemistry for this class of compounds. Compounds **4.21**$-$**4.27** were obtained as TFA salts with deprotection of the Boc group occurring during cleavage from the resin. This improved acid chloride method for the introduction of the spacers was also confirmed by the synthesis of non-amide DFO analogues of type **3** by simultaneously synthesizing **3.8** and **3.12** in 12 reaction vessels, each on the automated synthesizer along with the current library. The crude mass yields in each of the reaction vessels ranged from 98 to 121% with RP-HPLC purities ranging from 54 to 68%. The crude product from each vessel was appropriately combined and recrystallized from MeCN$-$H$_2$O (4:1) to afford pure **3.8** and **3.12**, respectively, in 46% overall yield. Thus, the fidelity of this library is 100%, on the basis of the MS (ESI) and HPLC data.

In the above library, when R = (CH$_2$)$_5$NH$_2$, the structures are equivalent to those produced in previous non-amide analogue libraries and DFO. Notably, when a = 1 and R = simple alkyl (Scheme 4), the resulting structure still had three chelating groups and was functionally equivalent to DFO itself and to the non-amide DFO analogue **1b**. This minimalist ligand lacks the pendant charged group seen in DFO (amino group) and the non-amide DFO (carboxyl group) that are not essential for iron binding. In fact, the terminal amino group of DFO is one site at which it is metabolized, and its removal may have an impact on the half-life of this DFO analogue. It was expected that an increase of log P for this compound may impact its biodistribution and iron excretion.

Reverse-Amide and Hybrid Analogues. Using β-alanine or an α-amino acid as a reagent instead of a dicarboxylic acid (e.g., succinic anhydride) as one of the spacers in the DFO synthetic sequence, we have prepared several "reverse-amide" analogues **5.1**$-$**5.22** with a variety of terminal groups (e.g., alkyl, carboxyalkyl, alkoxycarbonylalkyl, and aminoalkyl). The Fmoc strategy was compatible with the acid-labile protecting scheme used for permanent protection (linker and *O-tert*-butyl) during the synthesis. The Fmoc-β-

alanine and Fmoc-α-amino acids were coupled to the O-protected hydroxylamine residue **5.a** using HATU/DIPEA in DMA. After removal of the Fmoc group with piperidine, the resulting amine **5.b** was coupled to bromocarboxylic acid chloride, which in turn was reacted with *t*-BuO-NH-Ns in the presence of TMG to give **5.c**. The remainder of the synthesis is analogous to that described in Scheme 2. Another group of compounds, **6.1**$-$**6.19**, prepared from the same intermediate are hybrids of DFO and its non-amide analogue **1b**. These analogues lack one of the amide bonds found in DFO that are believed to decrease stability in vivo. Even though the synthesis of the reverse amide and hybrid analogues was illustrated using the nosyl resin **4.b** (Scheme 5), similar synthetic sequences were carried out using the CDI-activated Wang resin **2.a** (Scheme 2) and 6-bromohexanoic acid-derivatized Wang resin[33] of the type **3.a** (Scheme 3) through the intermediacy of **2.d** and **3.c**, respectively, resulting in the formation of the analogues with terminal amino and carboxyl groups.

A complex library of 90 analogues with various amino acids [Fmoc-Gly-OH, Fmoc-Sar-OH, Fmoc-Aib-OH, Fmoc-Ala-OH, Fmoc-MeAla-OH, Fmoc-Pro-OH, Fmoc-Lys-(Fmoc)-OH, Fmoc-Dpr(Fmoc)-OH, and Fmoc-β-Ala-OH], alcohols (MeOH, EtOH, *n*-PrOH, and *n*-BuOH), and acid chlorides (6-bromohexanoyl- and 8-bromooctanoyl-) was designed. Twenty-two reverse-amide analogues and 19 hybrid analogues of DFO were synthesized as depicted in Tables 4 and 5, respectively, indicating a 45% success with this library based on the RP-HPLC and MS (ESI) data.

It should be noted here that Fmoc-β-alanine worked best in the coupling reactions. In the case of Fmoc-α-amino acids, coupling was found to be more difficult, resulting mainly in deleted sequences. One other side reaction observed with these amino acids was cleavage at the hydroxamate moiety (coupling site), probably occurring during the prolonged exposure to TFA necessary for the deprotection of *tert*-butyl groups. The extent of deletion vs cleavage was highly dependent on the structure of both substrate and Fmoc-amino acid. When the coupling site was $-$N(O*t*-Bu)H, only Gly gave the desired products. While deletion was the major reaction pathway with Aib, MeAla, and Pro, cleavage was dominant with Sar, Dpr, and Lys. When the coupling site was $-$ON(R)H, desired products were isolated with Pro, also; however, deletion was observed with Aib, but cleavage was the preferred mode of reaction with Sar and Lys. Even with Fmoc-β-alanine, an additional β-Ala moiety was observed in some products. Even though compounds **5.3**, **5.4**, **5.7**, and **5.8** do not contain either β-Ala or Pro moiety, they are grouped with reverse-amide analogues simply for convenience. Similarly, compounds **6.5**$-$**6.9** are different alkyl esters of the non-amide DFO analogue **1b** synthesized for comparison purposes.

To avoid harsh reaction conditions for the deprotection of *tert*-butyl group, DMB protection for the hydroxamate was utilized in the synthesis of the hybrid analogues of the type **6** in which the amino acid is Fmoc-β-alanine (Scheme 6). The DMB group can be removed under relatively mild conditions (5% TFA in DCM), as shown in the synthesis of hydroxamic acids in solution[34] and from certain phenolic

Scheme 4. Solid-Phase Synthesis of *C*-Terminal Modified Non-Amide DFO Analogues[a]
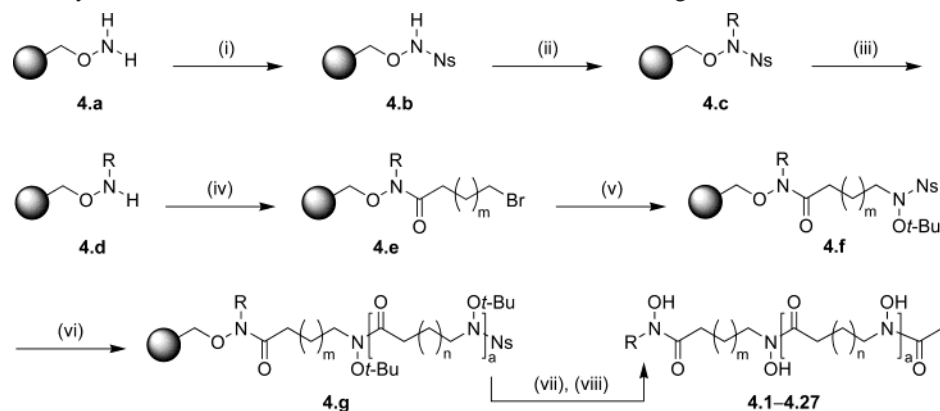
[a] Reagents and conditions: (i) NsCl, 2,6-lutidine, DCE, 4 h; (ii) ROH, Ph$_3$P, DIAD, THF, 37 °C, 4 h; (iii) HS(CH$_2$)$_2$OH, DBU, DMF, 30 min ($\times$ 2); (iv) bromocarboxylic acid chloride, DIPEA, DCE, 4 h; (v) *t*-BuO$-$NH$-$Ns, TMG, DMF, 50 °C, 6 h; (vi) repeat steps (iii) through (v) twice if needed; (vii) repeat step (iii); Ac$_2$O, DIPEA, DCE, 6 h; (viii) TFA$-$TIS$-$DCM (18:1:1), 2 h, filter, and then 15 h.

Table 3. RP-HPLC Purity and Masses Found for *C*-Terminal Modified Non-Amide DFO Analogues **4**[a]

| product	R	m	n	a	MW calcd	MW found[b]	purity[c], (%)
4.1[d]	Me	3	3	2	476	515 (M + K)$^+$	68
4.2	Me	3	5	2	532	533 (M + H)$^+$	50
4.3	Me	5	5	2	560	561 (M + H)$^+$	60
4.4	Me	3	3	1	347	348 (M + H)$^+$	50
4.5	Me	3	5	1	375	376 (M + H)$^+$	56
4.6	Me	5	3	1	375	376 (M + H)$^+$	55
4.7	Me	5	5	1	403	404 (M + H)$^+$	58
4.8[d]	Et	3	3	2	490	513 (M + Na)$^+$	78
4.9	Et	3	5	2	546	547 (M + H)$^+$	58
4.10	Et	5	5	2	574	575 (M + H)$^+$	61
4.11	Et	3	3	1	361	362 (M + H)$^+$	59
4.12	Et	3	5	1	389	390 (M + H)$^+$	53
4.13	Et	5	3	1	389	390 (M + H)$^+$	66
4.14	Et	5	5	1	417	418 (M + H)$^+$	67
4.15	Bn	3	5	2	608	609 (M + H)$^+$	54
4.16	Bn	5	5	2	636	637 (M + H)$^+$	75
4.17	Bn	3	3	1	423	424 (M + H)$^+$	53
4.18	Bn	3	5	1	451	452 (M + H)$^+$	53
4.19	Bn	5	3	1	451	452 (M + H)$^+$	55
4.20	Bn	5	5	1	479	480 (M + H)$^+$	56
4.21[d]	(CH$_2$)$_5$NH$_2$	3	3	2	547	548 (M + H)$^+$	59
4.22	(CH$_2$)$_5$NH$_2$	3	5	2	603	604 (M + H)$^+$	45
4.23	(CH$_2$)$_5$NH$_2$	5	5	2	631	632 (M + H)$^+$	48
4.24	(CH$_2$)$_5$NH$_2$	3	3	1	418	419 (M + H)$^+$	54
4.25	(CH$_2$)$_5$NH$_2$	3	5	1	446	447 (M + H)$^+$	57
4.26	(CH$_2$)$_5$NH$_2$	5	3	1	446	447 (M + H)$^+$	55
4.27	(CH$_2$)$_5$NH$_2$	5	5	1	474	475 (M + H)$^+$	60

[a] The crude mass yields were in the range 88$-$119% with respect to the initial loading of the nosyl resin (0.91 mmol/g). [b] In MS (ESI$^-$), the molecular ions corresponded to either (M $-$ H)$^-$ or (M + TFA)$^-$. [c] Purity was determined from the relative peak areas (%) of HPLC chromatograms (0$-$100% B/10 min). [d] Synthesized in polypropylene tubes and obtained in 80$-$86% crude yield on 0.06 mmol scale.

groups on the solid phase.[35] The resin-bound amine **7.a** was treated with nosyl chloride, and the resulting mononosyl derivative was alkylated with methyl *p*-toluenesulfonate in the presence of nonionic base 7-methyl-1,5,7-triazabicyclo-[4.4.0]dec-5-ene (MTBD) to give **7.b**. For the compounds in which R$_2$ = H, this N-activation and methylation steps were avoided. The remainder of the synthesis was analogous to that of **6**; however, since reactions were carried out in IRORI MiniKans, much longer reaction times were required.

The DMB protecting group was removed on-resin using 1% TFA containing 5% thioanisole scavenger. Subsequent cleavage from the resin was effected with TFA$-$DCM (4:1) to give the final products **7.1**$-$**7.16**.

All 16 possible compounds (R$_1$ = Me, Et; R$_2$ = H, Me; m = n = 3, 5) were synthesized and characterized (Table 6). The crude mass yields are relatively lower when compared to the other libraries, possibly due to loss of resin from the MiniKans as the synthesis progressed. However, on the basis of HPLC data, purer compounds were obtained.

Measurement of Affinity for Ferric Ion. To assess the impact of structural variation of DFO analogues on ferric-ion affinity, a high-throughput assay was developed. The assay developed used an optimized version of the competitive spectrophotometric method reported by Schwyn and Neilands.[36] The original method used chrome azurol S (CAS), with an extinction coefficient (ϵ) of 100 000 M^{-1} cm^{-1} at 630 nm. The addition of a strong iron chelator, such as DFO, resulted in the release of CAS with a concomitant reduction in absorbance. By plotting absorbance vs concentration for a series of chelators at four or more concentrations, linear relations were obtained whose slopes served as a relative measure of iron affinities. However, in our preliminary studies, we found that CAS could not reproducibly distinguish between DFO and other tight iron-binding test ligands, such as ethylenediaminetetraacetic acid (EDTA), *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA), *trans*-1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid (CDTA), and *N,N′*-bis(2-hydroxybenzyl)ethylenediamine-*N,N′*-diacetic acid (HBED), even though the range of their calculated log K_{eff} values spanned 5 orders of magnitude at this pH. In addition, this assay was performed at pH 5.6, and the potential therapeutic application of the DFO analogues required a more physiologically relevant pH. To extend the range of log K_{eff} that could be assessed and to work at a physiologically relevant pH (7.0), other spectrophotometric reagents were evaluated. We found that 8-hydroxyquinoline-5-sulfonic acid (sulfoxine) proved to be the most robust under our conditions. Ligand pK_a's and iron binding constants for sulfoxine were taken from Smith and Martell.[37] Preliminary speciation calculations for pH 7 solutions containing 7.5 $\times$ 10^{-6} M total iron and 7.5 $\times$ 10^{-5}

Scheme 5. Solid-Phase Synthesis of Reverse-Amide and Hybrid DFO Analogues[a]
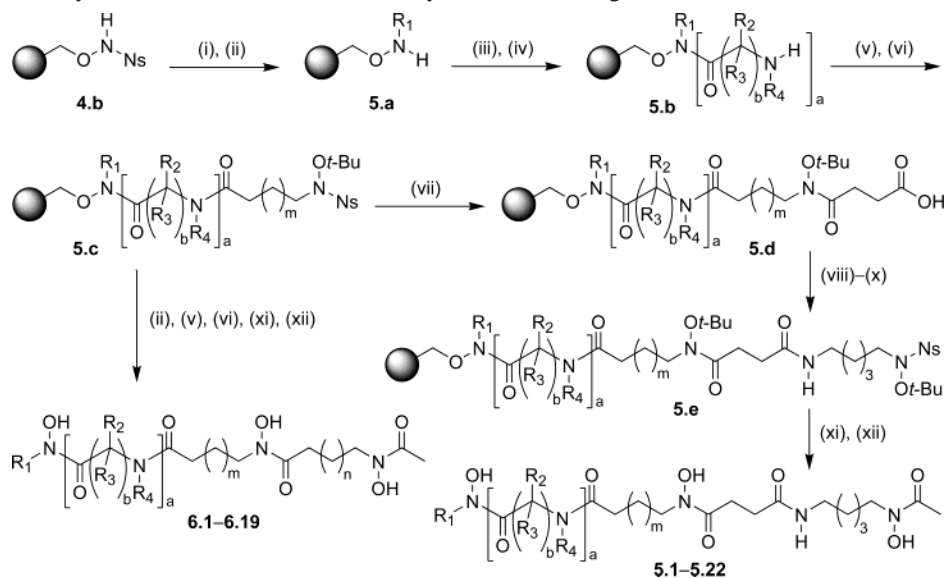
[a] Reagents and conditions: (i) R_1OH, Ph_3P, DIAD, THF, 37 °C, 4 h or R_1Br, DBU, DMF, 50 °C, 6 h; (ii) $HS(CH_2)_2OH$, DBU, DMF, 30 min ($\times$ 2); (iii) Fmoc-amino acid, HATU, DIPEA, DMA, 2 $\times$ 4 h; (iv) 25% piperidine in DMF, 3 and 15 min; (v) bromocarboxylic acid chloride, DIPEA, DCE, 4 h; (vi) t-BuO$-$NH$-$Ns, TMG, DMF, 50 °C, 6 h; (vii) repeat step (ii) succinic anhydride, DMAP, DMA, 50 °C, 6 h; (viii) CDI, THF$-$DMA (4:1), 2 h; (ix) $H_2N(CH_2)_5OH$, DIPEA, DMA, 12 h; (x) t-BuO$-$NH$-$Ns, Ph_3P, DIAD, THF, 37 °C, 4 h; (xi) repeat step (ii); Ac$_2$O, DIPEA, DCE, 6 h; (xii) TFA$-$TIS$-$DCM (18:1:1), 2 h, filter, and then 18 h.

Table 4. RP-HPLC Purities and Masses Found for Reverse-Amide DFO Analogues **5**[a]

product	R_1	a	b	R_2	R_3	R_4	m	MW calcd	MW found[b]	purity,[c] (%)
5.1	$(CH_2)_5NH_2$	1	2	H	H	H	3	560	561 (M + H)$^+$	72
5.2	$(CH_2)_5NH_2$	1	2	H	H	H	5	589	590 (M + H)$^+$	54
5.3	$(CH_2)_5NH_2$	0	0				3	489	490 (M + H)$^+$	41
5.4	$(CH_2)_5NH_2$	0	0				5	517	518 (M + H)$^+$	57
5.5	$(CH_2)_5NH_2$	1	1	H	H	H	3	546	547 (M + H)$^+$	61
5.6	$(CH_2)_5NH_2$	1	1	H	H	H	5	574	575 (M + H)$^+$	46
5.7	$(CH_2)_5CO_2H$	0	0				3	518	519 (M + H)$^+$	35
5.8	$(CH_2)_5CO_2H$	0	0				5	546	547 (M + H)$^+$	44
5.9	$(CH_2)_5CO_2H$	1	1	H	H	H	3	575	576 (M + H)$^+$	41
5.10	$(CH_2)_5CO_2H$	1	1	H	H	H	5	603	604 (M + H)$^+$	34
5.11	$(CH_2)_5CO_2Me$	1	2	H	H	H	3	603	604 (M + H)$^+$	40
5.12	$(CH_2)_5CO_2Et$	1	2	H	H	H	3	617	618 (M + H)$^+$	41
5.13	$(CH_2)_5CO_2n$-Pr	1	2	H	H	H	3	631	632 (M + H)$^+$	41
5.14	$(CH_2)_5CO_2n$-Bu	1	2	H	H	H	3	645	668 (M + Na)$^+$	52
5.15	Me	1	2	H	H	H	3	489	512 (M + H)$^+$	29
5.16	Me	1	2	H	$-(CH_2)_3-$		5	543	544 (M + H)$^+$	48
5.17	Et	1	2	H	H	H	3	503	526 (M + Na)$^+$	48
5.18	Et	1	2	H	$-(CH_2)_3-$		5	557	558 (M + H)$^+$	46
5.19	n-Pr	1	2	H	H	H	3	517	540 (M + Na)$^+$	45
5.20	n-Pr	1	2	H	$-(CH_2)_3-$		5	571	572 (M + H)$^+$	47
5.21	n-Bu	1	2	H	H	H	3	531	532 (M + H)$^+$	47
5.22	n-Bu	1	2	H	$-(CH_2)_3-$		5	585	586 (M + H)$^+$	58

[a] The crude mass yields were over 100% in most of the cases with respect to the initial loading of the nosyl resin (0.89 mmol/g). [b] In MS (ESI$^-$), the molecular ions corresponded to either (M $-$ H)$^-$ or (M + TFA)$^-$. [c] Purity was determined from the relative peak areas (%) of HPLC chromatograms (0$-$100% B/10 min).

M total ligand showed that iron would be present exclusively as the Fe(L$_2$)(OH) species (ϵ = 5800 M^{-1}cm^{-1} at 570 nm) in the sulfoxine solution. The effective binding constant (formal binding constant defined as β_{ijk} = [Fe$_i$L$_j$H$_k$]/[Fe]^i-[L]^j[H]^k) and pM (defined as $-$log of the concentration of the unchelated, hexaaquo ferric ion) were log β_{12-1} = 21.9 and pM = 18.6 for the sulfoxine complex. Similar calculations for DFO yielded log K_{eff} = 24.5 and pM = 25.2.

To ensure that the solutions are in equilibrium at the time of assay, test solutions were prepared in two ways: prefor-

mation of the sulfoxine$-$Fe complex with subsequent addition of the test ligand and, alternatively, preformation of the ligand$-$Fe complex with subsequent addition of sulfoxine. The results from both preparation methods must agree to verify that equilibrium has been reached. This method was adapted for 96-well microtiter plates with a plate reader. The limiting factor in the time required for analysis is the sample preparation, which was reduced by conducting the assay at a single ligand concentration. Instead of quantifying the iron affinity of the ligand as a slope (requiring several

Table 5. RP-HPLC Purities and Masses Found for Hybrid DFO Analogues **6**[a]

product	R_1	a	b	R_2	R_3	R_4	m	n	MW calcd	found[b]	purity,[c] (%)
6.1	$(CH_2)_5NH_2$	1	2	H	H	H	3	3	489	490 $(M + H)^+$	57
6.2	$(CH_2)_5NH_2$	1	2	H	H	H	3	5	517	518 $(M + H)^+$	58
6.3	$(CH_2)_5CO_2H$	1	2	H	H	H	3	3	518	519 $(M + H)^+$	43
6.4	$(CH_2)_5CO_2H$	1	2	H	H	H	3	5	546	547 $(M + H)^+$	37
6.5	$(CH_2)_5CO_2Me$	0	0				3	3	461	484 $(M + Na)^+$	63
6.6	$(CH_2)_5CO_2Et$	0	0				3	3	475	498 $(M + Na)^+$	57
6.7	$(CH_2)_5CO_2n\text{-}Pr$	0	0				3	3	489	490 $(M + H)^+$	57
6.8	$(CH_2)_5CO_2n\text{-}Bu$	0	0				3	3	503	526 $(M + Na)^+$	64
6.9	$(CH_2)_5CO_2Me$	1	2	H	H	H	3	5	560	561 $(M + H)^+$	48
6.10	$(CH_2)_5CO_2Et$	1	2	H	H	H	3	5	574	575 $(M + H)^+$	57
6.11	$(CH_2)_5CO_2n\text{-}Pr$	1	2	H	H	H	3	5	588	611 $(M + Na)^+$	48
6.12	$(CH_2)_5CO_2n\text{-}Bu$	1	2	H	H	H	3	5	602	603 $(M + H)^+$	51
6.13[d]	Me	1	2	H	H	H	3	5	446	447 $(M + H)^+$	52
6.14	Me	1	1	H	$-(CH_2)_3-$		5	5	500	501 $(M + H)^+$	75
6.15[e]	Et	1	2	H	H	H	3	5	460	461 $(M + H)^+$	54
6.16	Et	1	1	H	$-(CH_2)_3-$		5	5	514	515 $(M + H)^+$	69
6.17	$n\text{-}Pr$	1	1	H	$-(CH_2)_3-$		5	5	528	529 $(M + H)^+$	69
6.18	$n\text{-}Bu$	1	2	H	H	H	3	5	488	489 $(M + H)^+$	35
6.19	$n\text{-}Bu$	1	1	H	$-(CH_2)_3-$		5	5	542	565 $(M + Na)^+$	34

[a] The crude mass yields were over 100% in most of the cases with respect to the initial loading of the nosyl resin (0.89 mmol/g). [b] In MS (ESI⁻), the molecular ions corresponded to either $(M - H)^-$ or $(M + TFA)^-$. [c] Purity was determined from the relative peak areas (%) of HPLC chromatograms (0−100% B/10 min). [d] Same as compound **7.2**. [e] Same as compound **7.10**.

Scheme 6. Solid-Phase Synthesis of Hybrid DFO Analogues[a]
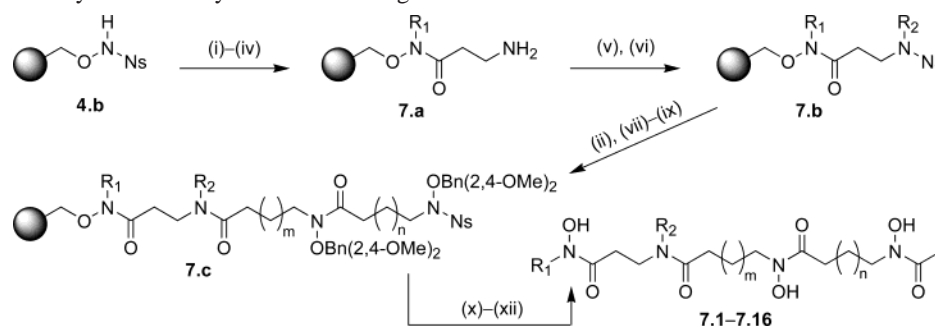
[a] Reagents and conditions: (i) R_1OH, Ph_3P, DIAD, THF, 37 °C, 4 h (× 2); (ii) $HS(CH_2)_2OH$, DBU, DMF, 1 h (× 2); (iii) Fmoc-β-Ala-OH, HOAt, HATU, DIPEA, DMA, 10 h; (iv) 20% piperidine in DMF, 6 and 40 min; (v) NsCl, 2,6-lutidine, DCE, 8 h; (vi) MeOTs, MTBD, DMF, 50 °C, 8 h; (vii) bromocarboxylic acid chloride, DIPEA, DCE, 13 h; (viii) 2,4-$(MeO)_2$BnO−NH−Ns, TMG, DMF, 50 °C, 12 h; (ix) repeat steps (ii), (vii), and (viii); (x) repeat step (ii); Ac_2O, DIPEA, DCE, 12 h; (xi) 1% TFA-5% thioanisole in DCM, 1 h (× 2); (xii) TFA−DCM (4:1), 2 h.

measurements at different concentrations), the amount of iron stripped by the unknown ligand was expressed as a percentage. This required only one measurement, $[A_0 - A]/[A_0] \times 100$, where A_0 is the absorbance of the initial sulfoxine−Fe complex, and A is the absorbance of the solution after addition and equilibration of uncharacterized ligand. Typically, the reproducibility of results was ±3%.

Most of the library compounds were screened in the assay, and the relative binding affinities of the selected compounds, measured as a percentage of iron stripped from the sulfoxine−Fe(III) complex, are given in Table 7. In all the binding assays, DFO (**1a**) and non-amide analogue (**1b**) were used as controls to determine the validity of the assay results. The DFO synthesized in the library, **2.1**, displayed an affinity to Fe(III) similar to the control DFO (**1a**). In the case of DFO variants, all of the structural modifications resulted in compounds with decreased iron-binding capabilities. For example, seemingly simple substitution of glutaric acid for succinic acid, as in **2.6**, significantly decreased the affinity for iron (34% displacement of iron). The lowest activity was seen for **2.11** (10%), in which all the spacers were modified,

whereas the greatest activity was found for **2.25** (49%). Other compounds with relatively higher affinities include **2.14** (47%) and **2.26** (44%), which differ from **2.25** only at the first dicarboxylic acid spacer. Thus, deviation from the DFO structure seems to have an adverse effect on the Fe(III) affinities of this class of compounds.

The higher-affinity compounds among non-amide analogues all contain an octanoic acid spacer in at least two positions, as shown by iron displacement by **3.8** (28%) and **3.12** (32%). In the case of C-terminal alkylated hydroxamates **4**, when $a = 1$, the structures still contain three hydroxamate groups necessary for the formation of hexadentate complexes with Fe(III). When R = aminopentyl, these compounds could be considered as equivalent to non-amide analogues with terminal amino groups instead of carboxyl groups. Substitution of each hexanoic acid spacer with an octanoic acid spacer increases the ability for iron removal from sulfoxine (∼16% for **4.4** and 31% for **4.7**). Although the relative binding affinities were found to be highly dependent on the length of the spacer between the hydroxamates, the nature of the R group has little or no effect (∼26% for **4.14** with R

Table 6. RP-HPLC Purities and Masses Found for Hybrid DFO Analogues **7**[a]

product	R_1	R_2	m	n	MW calcd	MW found[b]	purity[c], (%)
7.1	Me	H	3	3	418	441 (M + Na)$^+$	76
7.2[d]	Me	H	3	5	446	469 (M + Na)$^+$	65
7.3	Me	H	5	3	446	469 (M + Na)$^+$	65
7.4	Me	H	5	5	474	497 (M + Na)$^+$	58
7.5	Me	Me	3	3	432	455 (M + Na)$^+$	78
7.6	Me	Me	3	5	460	483 (M + Na)$^+$	74
7.7	Me	Me	5	3	460	483 (M + Na)$^+$	77
7.8	Me	Me	5	5	488	511 (M + Na)$^+$	71
7.9	Et	H	3	3	432	455 (M + Na)$^+$	69
7.10[e]	Et	H	3	5	460	483 (M + Na)$^+$	63
7.11	Et	H	5	3	460	483 (M + Na)$^+$	67
7.12	Et	H	5	5	488	511 (M + Na)$^+$	61
7.13	Et	Me	3	3	446	469 (M + Na)$^+$	82
7.14	Et	Me	3	5	474	497 (M + Na)$^+$	75
7.15	Et	Me	5	3	474	497 (M + Na)$^+$	78
7.16	Et	Me	5	5	502	525 (M + Na)$^+$	75

[a] The crude mass yields were in the range 42−97% with respect to the initial loading of the resin (0.91 mmol/g). [b] In MS (ESI$^-$), the molecular ions corresponded to (M + TFA)$^-$. [c] Purity was determined from the relative peak areas (%) of HPLC chromatograms (0−100% B/10 min). [d] Same as compound **6.13**. [e] Same as compound **6.15**.

= Et and 30% for **4.27** with R = aminopentyl). This R group is then a good handle to adjust log *P* and vary the bioavailability without harming the binding affinity. The compounds generally failed to equilibrate when R = Bn (percent iron removed from pfS and pfL are very different), and reliable results were not obtained. An additional spacer leading to compounds with four hydroxamate groups (*a* = 2) increased affinity for iron (∼43% for **4.2** and 23% for **4.5**) and followed similar general trends described above. However, once two octanoic acid spacers were in place, no further improvements were noticed in the affinity of the compounds (∼42% for **4.22** and 41% for **4.23**).

Reverse-amide analogues **5.1**−**5.22** containing a variety of terminal groups (e.g., alkyl, carboxyalkyl, alkoxycarbonylalkyl, and aminoalkyl) are most DFO-like compounds, both in structural similarities and Fe(III)-binding properties. The major difference is the replacement of the first succinic acid and aminopentanol spacers with an amino acid and alkanoic acid spacers, respectively. For the amino-terminal compounds, both hexanoic and octanoic acid spacer-containing compounds **5.1** and **5.2** displayed identical iron-removal abilities (53% each). However, replacement of hexanoic acid with octanoic acid spacer in amideless carboxyl-terminal compounds resulted in increased iron affinity (∼31% for **5.7** and 59% for **5.8**). Among the compounds with terminal (alkoxycarbonyl)hexyl group **5.11**−**5.14**, the propyl ester **5.13** (53% displacement) is comparable to that of amino-terminal compound **5.1**, and all other esters showed a slightly lower affinity for iron (44−46% displacement). Among simple alkyl terminal analogues **5.15**−**5.22**, all compounds containing the β-alanine spacer displayed similar abilities for the removal of iron from sulfoxine (57−65%), and the proline-containing compounds were somewhat inferior in the binding assay (41−47%).

The chimeric analogues **6** and **7** are hybrids of reverse-amide and non-amide analogues with the β-alanine spacer

at the first hydroxamate moiety. Insertion of a β-alanine spacer into **4.21** leads to **6.1**, which also increases the ability to displace iron from sulfoxine from 23 to 44%. This is equivalent to **4.22** in terms of iron-binding affinity, and the combination of the spacers β-alanine and hexanoic acid in succession seems to duplicate the octanoic acid spacer. Incorporation of β-alanine at the first hydroxamate moiety of the esters of the type **6.5**−**6.8** (19−25% displacement) led to compounds **6.9**−**6.12**, with slightly increased displacement properties (33−41%). Even the simplest analogues in this series, **6.13** (R = Me) and **6.15** (R = Et), removed 56 and 52%, respectively, of the iron from the sulfoxine complex. Somewhat cleaner samples of these compounds, **7.2** and **7.10** (IRORI library), displayed a lower affinity for iron (36 and 34% displacement, respectively). This may be due to the removal of the shorter-sequence hydroxamate impurities, which might have positively contributed to the removal of iron. These compounds contain additional β-alanine moiety at the first hydroxamate moiety when compared to **4.5** and **4.12** (18% displacement of iron), which resulted in increased iron binding. Among this class of chimeric compounds, **7.2** and **7.10**, containing a β-alanine−hexanoic acid−octanoic acid combination of the spacers, were found to be superior in the binding assay when compared to all other combinations of hexanoic and octanoic acid spacers. *N*-Methylated versions of these compounds, **7.6** and **7.14**, exhibited diminished iron-binding properties, with 24 and 23% of the iron removed from the sulfoxine complex.
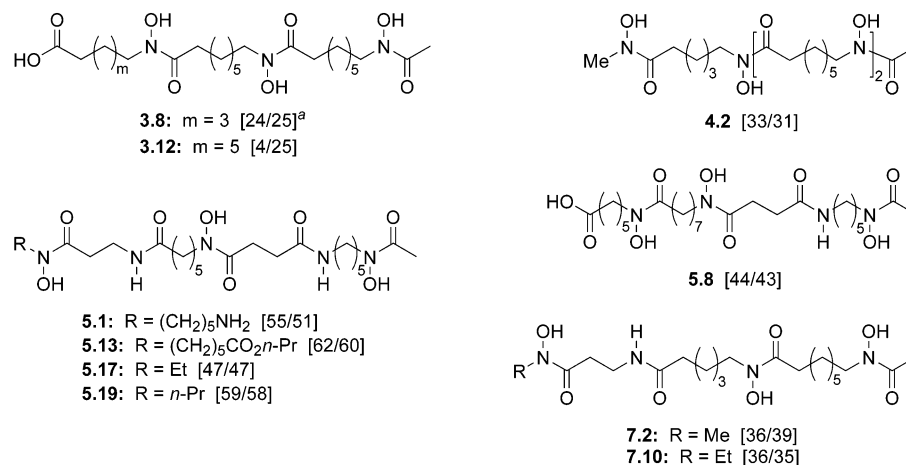
Scale-Up Synthesis. Some of the compounds with high relative Fe(III) binding affinity were selected and scaled up in order to obtain enough material for in vitro permeability studies and screening in different in vivo animal models of iron-overload disease. These include C-terminal methylated non-amide analogue **4.2**, reverse-amide analogues **5.1**, **5.13**, **5.17**, and **5.19**, and the hybrid analogues **5.8**, **7.2**, and **7.10**, possessing a wide variety of terminal groups (Figure 2). Synthesis was carried out using 0.55 mmol of the appropriate starting resin, and each compound was synthesized in two reaction vessels (Advanced ChemTech 496 Ω MOS, 16-reaction-vessel block), which was later consolidated. The substrate was bromohexanoic acid-derivatized Wang resin of type **3.a** for **5.8** and nosyl resin **4.b** for the rest of the compounds. The detailed synthetic methodology was already described in previous examples. DMB was used as the O-protecting group in synthesis of succinic acid spacer-containing compounds **5**, and *tert*-butyl was used in the synthesis of **4.2**, **7.2**, and **7.10**. After the synthesis, the products were cleaved from the resin and purified by multiple triturations of the residue with acetonitrile. The overall yields of the products ranged from 18 to 33%, with RP-HPLC purities of 74−95%.

Lower relative purities of compounds **5.1** (76%) and **5.17** (74%) were due to incomplete Mitsunobu reaction of *O*-(2,4-dimethyoxybenzyl)-*N*-nosylhydroxylamine [2,4-(MeO)$_2$BnO-NH-Ns] with aminopentanol moiety during the introduction of the last hydroxamate unit. Minor impurities in these compounds were identified as the acetates of the aminopentanol moiety on the basis of LC/MS data. All products were characterized by LC/MS, ^{1}H NMR, and HRMS. The Fe(III)

Table 7. Relative Iron Affinity of the Selected Analogues (**2**−**7**) in Sulfoxine Assay

compd	% Fe removed pfS^a (pfL)^b	compd	% Fe removed pfS^a (pfL)^b	compd	% Fe removed pfS^a (pfL)^b
1a	70 (68)	**4.4**	16 (20)	**5.13**	53 (53)
1b	12 (11)	**4.5**	23 (25)	**5.17**	57 (59)
2.1^c	67 (65)	**4.7**	31 (32)	**5.19**	62 (63)
2.6	34 (34)	**4.14**	26 (30)	**5.21**	65 (65)
2.11	10 (12)	**4.22**	42 (44)	**6.1**	44 (45)
2.14	47 (46)	**4.23**	41 (39)	**6.13**	56 (58)
2.25	49 (49)	**4.27**	30 (32)	**6.15**	52 (52)
2.26	44 (42)	**5.1**	53 (50)	**7.2**^{d}	36 (38)
3.8	28 (30)	**5.2**	53 (51)	**7.6**	24 (27)
3.12	32 (33)	**5.7**	31 (31)	**7.10**^{e}	34 (36)
4.2	43 (46)	**5.8**	59 (57)	**7.14**	23 (29)

^{a} pfS: preformation of the sulfoxine−Fe (III) complex with subsequent addition of the test ligand. ^{b} pfL: preformation of the ligand−Fe(III) complex with subsequent addition of sulfoxine. ^{c} DFO synthesized as the control. ^{d} Same as **6.13**. ^{e} Same as **6.15**.

3.8: m = 3 [24/25]^a
3.12: m = 5 [4/25]

4.2 [33/31]

5.1: R = (CH$_2$)$_5$NH$_2$ [55/51]
5.13: R = (CH$_2$)$_5$CO$_2$*n*-Pr [62/60]
5.17: R = Et [47/47]
5.19: R = *n*-Pr [59/58]

5.8 [44/43]

7.2: R = Me [36/39]
7.10: R = Et [36/35]

^{a}The numbers in the parentheses are % Fe removed expressed as pfS/pfL

Figure 2. Scale-up synthesis of selected DFO analogues and their Fe(III)-binding affinities in sulfoxine assay.

binding results from the sulfoxine assay were determined and listed in Figure 2. Also listed are previously purified non-amide analogues, **3.8** and **3.12**, obtained in 46% overall yield. Usually, there is good agreement in iron-binding affinity of these purified compounds with those of previous library compounds in the sulfoxine assay. In the case of **3.12**, iron transferred from the sulfoxine complex is only 4%, as compared to 25% of the iron retained by the compound in the presence of sulfoxine. This indicates that equilibrium was not reached during complex formation.

Conclusions

In summary, several focused combinatorial libraries of DFO analogues with a wide variety of terminal groups were synthesized via solid-phase methods. To assess the relative iron-binding affinities of library compounds, a high-throughput spectrophotometric screening assay based on competition with sulfoxine was developed. On the basis of the iron-binding results, some promising candidates were selected, synthesized on a larger scale, and fully characterized. These compounds are being evaluated in permeability studies in in vitro, cell-based assays for biodistribution in vivo, and in in vivo animal models of iron-overload diseases.

Experimental Section

General. Unless otherwise noted, all reagents were used as supplied. Wang resin (styrene−1% DVB copolymer, 100−200 mesh, 1.10 mequiv/g) was obtained from AnaSpec,

Inc. (San Jose, CA) and SynPhase Crowns [PS crown, O series, 4-(hydroxymethyl)phenoxyacetamido linker, 2.2 μmol] were purchased from Chiron Technologies Pty Ltd. (Clayton, Victoria, Australia). Solvents used in the reactions were anhydrous (sure seal) from Aldrich Chemical Co., Inc. (Milwaukee, WI), and others were of reagent or HPLC grade from Fisher Scientific (Pittsburgh, PA). Reactions were optimized in individual polypropylene tubes; filtrations and washings were carried out on a multiport vacuum manifold from P. J. Cobert Associates, Inc. (St. Louis, MO). Solvent evaporation was performed on an Atlas HT-4 centrifugal evaporator from GeneVac Ltd. (Ipswich, England). HPLC analyses were carried out on a Gilson (Middleton, WI) dual pump (model 306) system equipped with a liquid handler (model 215) and UV/vis-155 Detector using a Waters YMC ODS-AQ S5 120 Å 5-μm (4.6 × 50 mm) column. Compounds were injected using a single-gradient condition (0−90%B/10 min or 10−90% B/9 min or 0−100%B/10 min) using a flow rate of 2 mL/min and UV detection at 218 nm. Mobil phase A consisted of 0.10% TFA in H$_2$O, and mobile phase B consisted of 0.08% TFA in CH$_3$CN. Unless specified, NMR spectra were recorded in DMSO-d_6. Chemical shifts are expressed in parts per million (*d*) relative to TMS (*d* = 0) as an internal standard. Coupling constants (*J*) are reported in Hertz. Electrospray-ionization (ESI) mass spectra in either a negative or positive mode were obtained from Mass Consortium (San Diego, CA). LC/MS (ESI) data were obtained on a ThermoQuest AQA spectrometer

(Manchester, U.K.) using the above Waters YMC column running a single-gradient condition (100/0 of A/B to 0/100 in 10 min) with a flow rate of 0.50 mL/min and UV detection at 190−600 nm. Mobil phase A consisted of 0.10% TFA in H$_2$O, and mobile phase B consisted of CH$_3$CN. HRMS (FAB) data was obtained on a JEOL JMS-700 M instrument with PEG as the reference and NBA as the matrix. UV absorbance during the sulfoxine assay was measured using a FL 600 microplate fluorescence reader with KC4 processing software from Biotek (Winooski, VT).

Experimental Conditions for Resin-Bound Substrates and Reagents. Resin 2.c. Resin **2.a** (2.29 g, 0.96 mmol/g, 2.20 mmol),[25] prepared by CDI activation of Wang resin (2.0 g, 1.1 mmol/g, 2.2 mmol), was suspended in 20 mL of DMF in a Merrifield vessel, and DIPEA (0.575 mL, 3.30 mmol) was added. Then a solution of 5-amino-1-pentanol (1.14 g, 11.0 mmol) in DMF (5 mL) was added, and the suspension was heated and agitated for 24 h at 60 °C using heating tape coiled around the reaction vessel. After the suspension was cooled and the supernatants were removed, and the resin was washed with 30 mL each of DMF (×2), EtOH (×1), and DCM (×2) and then dried under high vacuum overnight. The resulting resin-bound alcohol **2.b** (2.26 g) was suspended in THF (25 mL) and agitated with *O-tert*-butyl-*N*-nosylhydroxylamine (1.81 g, 6.60 mmol),[21] Ph$_3$P (1.73 g, 6.60 mmol), and DIAD (1.30 mL, 6.60 mmol) at 37 °C for 4 h. Then the supernatants were removed, and the resin was washed with 30 mL each of THF (×2), DMF (×2), EtOH (×1), and DCM (×2) and finally dried under high vacuum overnight to give **2.c** (2.89 g; loading, 0.76 mmol/g). To determine the extent of reaction, 100 mg of the dry resin was subjected to cleavage with TFA−DCM (1:1), resulting in 5-[*N-tert*-butyloxy-*N*-(2-nitrophenylsulfonyl)]aminopentylamine as a TFA salt (35 mg, 95%): RP-HPLC 90% (t_R = 4.20 min); LC/MS (ESI) *m/z* 360 (M + H)$^+$.

Resin 3.a. Resin-bound bromide **3.a** (3.54 g; loading, 0.93 mmol/g) was prepared from Wang resin (3.0 g, 1.1 mmol/g, 3.3 mmol) according to a reported procedure[33] with slight modification (18 h reaction in DMF was changed to a two-cycle 1-h reaction in THF).

Resin 4.b. Resin **4.a** (4.11 g, 1.07 mmol/g, 4.40 mmol), prepared from Wang resin (4.0 g, 1.1 mmol/g, 4.4 mmol) according to a reported procedure,[31] was swelled with DMF (50 mL) in a Merrifield vessel and then washed with DCE (2 × 40 mL). The resin was suspended in DCE (45 mL), and 2,6-lutidine (3.85 mL, 33.0 mmol) was added. A solution of 2-nitrobenzenesulfonyl chloride (2.93 g, 13.2 mmol) in DCE (15 mL) was added, and the suspension was agitated for 4 h at room temperature. After filtration, the resin was washed successively with 50-mL portions of DCE (×2), DMF (×2), EtOH (×2), and CH$_2$Cl$_2$ (×2), which was then dried under high vacuum to give **4.b** (4.89 g; loading, 0.90 mmol/g). The reaction can also be carried out in DCE using pyridine as base or in pyridine as solvent without compromising the loading (typically 0.89−0.91 mmol/g) of the resin and the purity of the subsequent reaction products.

5-*N*-(*tert*-Butoxycarbonylamino)-1-pentanol. This compound was prepared from 5-amino-1-pentanol (60 mmol) in 83% yield (10.1 g) according to a reported procedure.[38]

8-Bromooctanoyl Chloride. Oxalyl chloride (11.2 mL, 128 mmol) was slowly added to a solution of 8-bromooctanoic acid (14.3 g, 64.0 mmol) in DCM (130 mL) containing catalytic DMF (0.124 mL, 2.5 mol %) while stirring at 0 °C and then warmed to r.t. over a period of 3 h. The solvent was removed on a rotary evaporator, and the residue was coevaporated with DCM (30 mL) and then dried under high vacuum overnight to give 15.0 g (97%) of a pale yellow oil (previously prepared using SOCl$_2$),[39] which was used without any further purification: IR (neat) ν_{max} = 1796 cm^{-1}.

6-Bromohexanoic Acid Propyl Ester. A solution of 6-bromohexanoyl chloride (4.21 mL, 27.5 mmol) in DCM (10 mL) was slowly added to 1-propanol (1.87 mL, 25.0 mmol), pyridine (2.42 mL, 30.0 mmol), and DMAP (0.153 g, 1.25 mmol) in DCM (40 mL) while stirring at ice-bath temperature. The pale yellow suspension was srirred at r.t. for 4 h and then treated with 2 N HCl (30 mL). The layers were separated, and the aqueous layer was further extracted with DCM (3 × 25 mL). The combined organic extracts were successively washed with 2 N HCl, H$_2$O, sat. NaHCO$_3$, H$_2$O, and brine (40 mL each) and then dried over Na$_2$SO$_4$. The solvent was removed, and the residue was distilled at reduced pressure to give 4.57 g (77%) of a colorless oil: bp 104−106 °C/3.1 mmHg; ^{1}H NMR (CDCl$_3$) 4.04 (t, 2H, *J* = 6.9), 3.42 (t, 2H, *J* = 6.6), 2.33 (t, 2H, *J* = 7.2), 1.93−1.84 (m, 2H), 1.71−1.45 (m, 6H), 0.95 (t, 3H, *J* = 7.2).

6-Bromohexanoic Acid Butyl Ester. This compound was prepared by substituting 1-butanol (2.28 mL, 25.0 mmol) for 1-propanol in the above procedure and 4.98 g (79%) of colorless oil was obtained: bp 112−115 °C/2.2 mmHg (lit.[40] bp 120−125 °C/0.1 mmHg); ^{1}H NMR (CDCl$_3$) 4.08 (t, 2H, *J* = 6.9), 3.42 (t, 2H, *J* = 6.9), 2.33 (t, 2H, *J* = 7.2), 1.93−1.84 (m, 2H), 1.71−1.32 (m, 8H), 0.94 (t, 3H, *J* = 7.2).

Experimental Conditions for Resin Reactions (Compounds 2.1−2.26, 4.1−4.27, 5.1−5.22, and 6.1−6.19). Compounds **2.1−2.26**, **4.1−4.27**, **5.1−5.22**, and **6.1−6.19** were synthesized using the Advanced Chemtech 496 Ω MOS System unless otherwise noted. To begin library synthesis, freshly prepared resin (either **2.c**, **3a** or **4.b**; 0.06 mmol) was loaded into individual wells within a 96-well format reaction block. The resin was swelled in DMF and washed with the solvent in which the reaction was going to be performed. Usually, 0.20 mL of the appropriate solvent was dispensed into each well to compensate for the dead volume. Typical washing cycles involved mixing the resin with 1.0−1.5 mL of the specified solvent at 600 rpm for 1 min and emptying the block for 4−5 min with N$_2$ at a pressure of 9 psi. After each reaction, the final washing cycle was always carried out with the solvent in which the next reaction was going to be carried out. The reagent solutions (anhydrous when possible) and solvents were delivered into the reaction wells by robotic arms (except during the cleavage of compounds from the solid support, which was done manually). All of the operations and reactions were performed under a N$_2$ atmosphere. During the course of the reactions, the reaction block was agitated at 600 rpm for the specified amount of time. End-capping with acetic anhydride and DIPEA was carried out in DMF after the synthesis of the monomer (protected form). After the completion of the synthesis, the

resins were dried (the reaction block was connected to the vacuum pump used for draining the solvents) prior to the cleavage.

End-Capping. In each case, after the monomer synthesis, the resin was reacted with acetic anhydride (0.50 M, 0.30 mL, 2.5 equiv) and DIPEA (1.0 M, 0.30 mL, 5.0 equiv) in DMF for 2 h at room temperature. The resin was washed with DMF (×2), EtOH (×1), and DMF (×2).

Deprotection of 2-Nitrobenzenesulfonyl Group. The resin-bound nosyl compound was agitated with a solution of 2-mercaptoethanol (0.40 M, 0.45 mL, 3 equiv) and DBU (0.80 M, 0.45 mL, 6 equiv) in DMF for 30 min at room temperature. The yellow colored solution was drained, and the resin was washed with EtOH and DMF (1 mL each). The reaction was repeated with fresh reagents as described above. Finally, the resin was washed with DMF (×2), EtOH (×1), and DMF or DCE (×2), depending upon the solvent for next reaction.

Coupling with Dicarboxylic Anhydrides. The resin-bound O-protected hydroxylamines were reacted with a solution of dicarboxylic acid anhydride (0.50 M, 0.60 mL, 5 equiv) in DMA containing DMAP (0.5 equiv) for 6−8 h at 50 °C. The resin was washed with DMF (×2), EtOH (×1), and DMF (×2).

Coupling with Dicarboxylic Acids. The resin-bound O-protected hydroxylamines were reacted with a solution of the active ester of the dicarboxylic acid of interest (0.25 M, 1.2 mL, 5 equiv), preformed in situ from dicarboxylic acid (5 equiv), HATU (5 equiv), and DIPEA (10 equiv) in DMA (1.2 mL), for 8 h at 50 °C. Resins were washed with DMF (×2), EtOH (×1), and DMF (×2).

Activation of Terminal Carboxylic Acids with CDI and Coupling of Amino Alcohols. Resin-bound terminal carboxylic acids were activated for coupling by reacting with CDI (0.50 M, 0.60 mL, 5 equiv) in THF−DMA (4:1) for 2 h at room temperature. After washing with DMA (×2), each of the acyl imdazolides was reacted with a solution of amino alcohol (0.50 M, 0.60 mL, 5 equiv) in DMA containing DIPEA (5 equiv) for 12 h at room temperature. The resin was washed with DMF (×2), EtOH (×1), and DMF (×2).

Mitsunobu Reaction of Resin-Bound Alcohols with *t*-BuO-NH-Ns. The resin-bound alcohol was agitated and heated with THF solutions of *O-tert*-butyl-*N*-nosylhydroxylamine (0.50 M, 0.48 mL, 4 equiv), Ph₃P (1.0 M, 0.24 mL, 4 equiv), and DIAD (1.0 M, 0.24 mL, 4 equiv) for 4 h at 37 °C. The resin was washed with THF (×2), DMF (×1), EtOH (×1), and DMF (×1).

Acetylation of Terminal O-Protected Hydroxylamines. The resin of interest was agitated with a solution of acetic anhydride (0.50 M, 0.60 mL, 5 equiv) and DIPEA or pyridine (1.0 M, 0.60 mL, 10 equiv) in DMF or DCE (as specified) for 6 h at room temperature. The resin was washed with DMF (×2), EtOH (×1), and DCE (×3).

Mitsunobu Reaction of Alcohols with Resin-Bound Nosylhydroxylamine. The resin-bound substrate was heated with THF solutions of the appropriate alcohol (0.75 M, 0.40 mL, 5 equiv) containing Et₃N (5 equiv), Ph₃P (1.0 M, 0.30 mL, 5 equiv), and DIAD (1.0 M, 0.30 mL, 5 equiv) for 4 h

at 37 °C. The resin was washed with THF (×2), DMF (×1), EtOH (×1), and DMF (×1).

Alkylation of Resin-Bound Nosylhydroxylamine with Alkyl Bromides. The resin-bound substrate was suspended in DMF (0.33 mL) and then heated with a solution of the appropriate 6-bromohexanoic acid alkyl ester (0.50 M, 0.48 mL, 4 equiv) and DBU (0.80 M, 0.15 mL, 2 equiv) in DMF for 6 h at 50 °C. The resin was washed with DMF (×2), EtOH (×1), and DMF (×2).

Acylation with Bromoacid Chlorides. The resin-bound substrate was reacted with DCE solutions of the appropriate bromoacid chloride (0.50 M, 0.48 mmol, 4.0 equiv) and DIPEA (1.0 M, 0.48 mmol, 8 equiv) in DCE for 4 h at room temperature. The resin was washed with DMF (×2), EtOH (×1), and DMF (×2).

Nucleophilic Displacement of Resin-Bound Alkyl Bromide with *t*-BuO-NH-Ns. The resin was agitated with an orange solution of DMF (0.90 mL) containing *O-tert*-butyl-*N*-nosylhydroxylamine (0.20 M, 3 equiv) and TMG (0.133 M, 2.0 equiv) at 50 °C for 6 h. The resin was washed with DMF (×2), EtOH (×1), and DMF (×2).

Coupling with Fmoc-Protected Amino Acids. The resin-bound O-protected hydroxylamines were shaken with a solution of the appropriate Fmoc-amino acid (0.50 M, 0.48 mL, 4 equiv), HATU (0.50 M, 0.48 mL, 4 equiv), and DIPEA (1.0 M, 0.48 mL, 8 equiv) in DMA for 4 h at room temperature. The solution was drained, and the resin was washed with DMA (×2). The reaction was repeated with fresh reagents using half of the above quoted amounts. Finally, the resin was washed with DMF (×2), EtOH (×1), and DMF (×2).

Deprotection of Fmoc Group. The Fmoc-protected resin was agitated with a solution of 25% piperidine in DMF (1.0 mL) for 3 min at room temperature. The solution was drained, and the reaction was repeated with fresh reagents for 15 min. Finally, the resin was washed with DMF (×2), EtOH (×1), and DMF (×2).

Cleavage from Resin. The compounds were simultaneously cleaved from the resin by agitation of resin-bound compounds with a 1.5-mL solution of TFA−DCM (9:1) for 30 min to 1 h (with terminal amino or carboxyl groups) or TFA−TIS−DCM (18:1:1) for 2 h. After filtration, the resin was washed with 1.0 mL of the respective cleavage cocktail, and the combined solution was transferred to a closed screw-cap vial and left overnight (15−22 h) at room temperature. The solutions were transferred to glass tubes and evaporated to dryness. The residue was coevaporated with acetonitrile (2 × 1 mL) and then further dried under high vacuum overnight.

Experimental Conditions for Reactions on MiniKans (Compounds 7.1−7.16). Members of this library were synthesized in IRORI MiniKan polypropylene reactors using the AccuTag-100 combinatorial chemistry system. The nosyl resin **4.b** (0.06 g, 0.91 mmol/g, 0.055 mmol) was loaded into 16 separate MiniKans, each containing a discrete radio frequency tag. Subsequent chemical operations were carried out in round-bottom flasks under N₂, and sorting of the MiniKans was done by using the AccuTag system. After the addition of solvent or reagent solutions, air bubbles were

removed from the MiniKans by applying vacuum (10−20 mmHg) for 5−10 s. During the wash cycles, the MiniKans were stirred for 15 min with 25 or 50 mL of solvent for the sets of 8 or 16 MiniKans, respectively, and were collected by vacuum filtration. After finishing the wash cycles, the MiniKans were dried under vacuum (10−20 mmHg) for 30 min.

Mitsunobu Reaction on Resin-Bound Nosylhydroxylamine. A set of eight MiniKans was suspended in a 0.25 M solution of Ph$_3$P (1.64 g, 6.25 mmol) in THF (25 mL), vacuum-degassed, and flushed with N$_2$. The relevant alcohol (6.25 mmol) and DIAD (1.23 mL, 6.25 mmol) were added and stirred at 37 °C for 4 h. Each set of the MiniKans was washed separately with THF (×3), and the reaction was repeated with fresh reagents. Once again, each set of the MiniKans was washed separately with THF (×3) and then together with DMF (×1), EtOH (×1), and DCM (×2). End-capping was carried out by stirring the set of 16 MiniKans with 0.40 M acetic anhydride (1.89 mL, 20.0 mmol) and 0.80 M DIPEA (6.95 mL, 40.0 mmol) in DMF (50 mL) for 3 h at room temperature. The MiniKans were washed with DMF (×1) and then alternately with EtOH and DCM (×3).

Deprotection of 2-Nitrobenzenesulfonyl Group. A set of 16 MiniKans was suspended in DMF (46.3 mL) and vacuum-degassed, and DBU (2.99 mL, 20.0 mmol) and 2-mercaptoethanol (0.70 mL, 10.0 mmol) were added and stirred for 1 h at room temperature. The yellow solution was removed, and the MiniKans were washed with DMF. The reaction was repeated with fresh reagents, and the MiniKans were washed with DMF (×1) and then alternately with EtOH and DCM (×4).

Coupling with *N*-Fmoc-*β*-Alanine. A set of 16 MiniKans was suspended in DMA (46.5 mL) and vacuum-degassed, and *N*-Fmoc-*β*-alanine (3.11 g, 10.0 mmol), HOAt (1.36 g, 10.0 mmol), HATU (3.80 g, 10.0 mmol), and DIPEA (3.48 mL, 20.0 mmol) were added. After stirring the MiniKans for 10 h at room temperature, they were washed alternately first with EtOH and DMF (×2) and then with EtOH and DCM (×2).

Deprotection of Fmoc Group. Deprotection of the Fmoc group was carried out in two batches. The intermediates leading to secondary amide analogues were saved as Fmoc derivatives until subsequent N-methylation and deprotection of the nosyl group, leading to the rest of the analogues. Thus, a set of eight MiniKans was suspended in 20% piperidine in DMF (25 mL), vacuum-degassed, and stirred for 6 min at room temperature. The solution was decanted, fresh deprotection cocktail was added, and then the mixture was stirred an additional 40 min. The MiniKans were washed alternately with EtOH and DCM (×4).

Protection of Terminal Amine with Nosyl Group. A set of eight MiniKans containing the resin-bound terminal amine was suspended in DCE (15 mL) and vacuum-degassed, and 2,6-lutidine (1.46 mL, 12.5 mmol) and 2-nitrobenzenesulfonyl chloride (1.11 g, 5.00 mmol) in DCE (8.5 mL) were added. The MiniKans were agitated for 8 h at room temperature and then washed alternately with EtOH and DCM (×3).

N-Methylation of Nosyl-Protected Terminal Amine. A set of eight MiniKans containing the terminal nosyl-protected amine was suspended in DMF (25 mL) and vacuum-degassed, and methyl-*p*-toluenesulfonate (1.16 g, 6.25 mmol) and MTBD (0.479 g, 3.13 mmol) were added. The MiniKans were stirred for 8 h at 50 °C and washed alternately first with EtOH and DMF (×2) and then with EtOH and DCM (×2).

Acylation of Terminal Amine or O-Protected Hydroxylamine. A set of eight MiniKans was suspended in DCE (21.8 mL) and vacuum-degassed, and DIPEA (2.17 mL, 12.5 mmol) and the appropriate bromoacid chloride (6.25 mmol) were added. The MiniKans were stirred for 13 h at room temperature, and each set was washed separately with DCE (×2) and EtOH (×1) and then together alternately with EtOH (×1) and DCM (×2) in two cycles.

Nucleophilic Displacement of Terminal Alkyl Bromide. A set of 16 MiniKans was suspended in an orange-red solution of 0.20 M *O*-(2,4-dimethoxybenzyl)-*N*-nosylhydroxylamine (2.94 g, 8.00 mmol)[21] and 0.15 M TMG (0.752 mL, 6.00 mmol) in DMF (40 mL) and agitated for 12 h at 50 °C. The solution was decanted, and the MiniKans were washed with DMF (×2) and then alternately with EtOH (×1) and CH$_2$Cl$_2$ (×2) in two cycles.

Acetylation of Terminal O-Protected Hydroxylamines. A set of 16 MiniKans was suspended in DCE (44.4 mL) and vacuum-degassed, and DIPEA (4.35 mL, 25.0 mmol) and acetic anhydride (1.18 mL, 12.5 mmol) were added. The MiniKans were stirred for 12 h at room temperature and washed with DMF (×2) and then alternately with EtOH (×1) and DCM (×2) in two cycles.

Deprotection of 2,4-Dimethoxybenzyl Groups. A set of 16 MiniKans was suspended in 1% TFA/5% thioanisole in DCM (50 mL) and vacuum-degassed, and stirred for 1 h at room temperature. The pale-pink turbid solution was removed, the MiniKans were washed with DCM (×2), and the reaction was repeated with fresh reagents. Finally, the MiniKans were washed first alternately with EtOH (×1) and DMF (×2) and then with EtOH (×1) and DCM (×2) in two cycles. The resulting MiniKans were dried overnight under high vacuum.

Cleavage from Resin. The cleavage was done using a IRORI 96-vessel cleavage station (AccuCleave-96). The compounds were cleaved off the resin directly from the MiniKans by reacting with a 2.5-mL solution of TFA−DCM (4:1 v/v) for 2 h at room temperature. After filtration, the MiniKans were washed with cleavage cocktail (2.5 mL each), and the combined solutions were transferred to glass tubes and evaporated to dryness. The residue was coevaporated with acetonitrile (2 × 2 mL) and then further dried under high vacuum overnight to give **7.1**−**7.16** as pale brown solids.

Experimental Conditions for Reactions on SynPhase Crowns (Compounds 3.1−3.12). The synthesis of compounds **3.1**−**3.12** was performed on pins with an aminomethyl polystyrene grafted surface derivatized with a 4-(hydroxymethyl)phenoxyacetic acid linker (2.2 μmol loading per pin), which was mounted on a block in an arrangement and spacing corresponding to a 96-well Microtiter reaction plate

(kit from Chiron Technologies).[41] All pins were washed with DMF (×3), DCM (×3), and THF (×3) prior to the synthesis. The pin block was then lowered over a series of reaction plates to immerse the pins in the wells of the plates in order to perform the synthetic steps of interest. The removal of reaction solutions and rinses from the solid support was accomplished by physically lifting the pins out of the reaction solutions, which were retained in 96-well microtiter plates, and dipping the pins into rinse solutions. A typical washing cycle after each step consisted of washes with DMF (×3), EtOH (×2), DCM (×2), and DMF (×2).

Loading of Pins with Bromocarboxylic Acid. Each pin was reacted with a solution consisting of appropriate bromocarboxylic acid (0.25 M), DIC (0.25 M), and DMAP (0.012 M) in THF (0.2 mL per pin) for 1 h at room temperature. The reaction was repeated again with fresh reagents.

Nucleophilic Displacement of Terminal Alkyl Bromide with *t*-BuO-NH-Ns. A solution of *O-tert*-butyl-*N*-nosylhydroxylamine (0.20 M) and DBU (0.15 M) in DMF (0.2 mL per pin) was reacted with each pin in the block for 2 h at 50 °C.

Deprotection of 2-Nitrobenzenesulfonyl Group. Deprotection was accomplished by reacting each pin with a solution of mercaptoethanol (0.20 M) and DBU (0.40 M) in DMF (0.2 mL per pin) for 30 min. This process was repeated with fresh reagents.

Acylation of O-Protected Hydroxylamines with Bromocarboxylic Acid. A solution of the desired bromocarboxylic acid (0.25 M), HATU (0.25 M), and DIPEA (0.25 M) in DMF (0.2 mL per pin) was reacted with each pin for 4 h at room temperature.

Coupling of Acetic Acid to Terminal O-Protected Hydroxylamines Amine. A solution of acetic acid (0.25 M), HATU (0.25 M), and DIPEA (0.25 M) in DMF (0.2 mL per pin) was reacted with each pin for 4 h at room temperature.

Cleavage from Pins. A solution of TFA−DCM (9:1) was reacted with each pin (0.4 mL) for 3 h. After removal of the pins, the cleaved solutions were transferred to glass tubes and evaporated to dryness. The residue was coevaporated with acetonitrile (2 × 0.5 mL) and then dried under high vacuum.

Experimental Conditions for Compounds in Scale-Up Synthesis (Figure 2). Compounds **4.2**, **5.1**, **5.8**, **5.13**, **5.17**, **5.19**, **7.2**, and **7.10** were synthesized using the Advanced Chemtech 496 Ω MOS System according to the general procedures described above. A 16-well reaction block was used, and each compound was synthesized in two wells. The wells were loaded with Wang resin-bound bromide **3.a** (0.59 g, 0.93 mmol/g, 0.55 mmol) for **5.8** and the nosyl resin **4.b** (0.61 g, 0.90 mmol/g, 0.55 mmol) for the rest of the compounds. 2,4-(MeO)$_2$BnO-NH-Ns was used in the synthesis of succinic acid spacer containing compounds **5**, and *t*-BuO-NH-Ns was used in the synthesis of **4.2**, **7.2**, and **7.10** during the introduction of hydroxamate moieties (displacement of bromide/Mitsunobu reaction with alcohol). Acylation with bromoacid chlorides was carried out with pyridine as the base for the *β*-alanine spacer containing compounds. After the synthesis, the products were cleaved from the resin,

and each product was consolidated into one lot and dried under high vacuum. The residues were triturated with acetonitrile containing traces of methanol two or three times to give reasonably pure products for characterization. Compounds **3.8** and **3.12** were synthesized during other library synthesis, as mentioned before.

7,16,25-Trihydroxy-8,17,26-trioxo-7,16,25-triazaheptacosanoic Acid (3.8). Colorless powder; yield 0.167 g (46%); ^{1}H NMR 9.59 (br s, 1H, D$_2$O-exchangeable), 9.45 (br s, 2H, D$_2$O-exchangeable), 3.36 (t, 6H, J = 6.7), 2.22 (t, 4H, J = 7.4), 2.09 (t, 2H, J = 7.2), 1.87 (s, 3H), 1.48−1.29 (m, 12H), 1.22−1.04 (m, 14H); RP-HPLC 91% (t_R = 4.79 min); LC/MS (ESI) m/z 504 (M + H)$^+$. HRMS (FAB) m/z calcd for C$_{24}$H$_{46}$N$_3$O$_8$ (M + H)$^+$ 504.3285, found 504.3279.

9,18,27-Trihydroxy-10,19,28-trioxo-9,18,27-triazanonacosanoic Acid (3.12). Colorless powder; yield 0.176 g (46%); ^{1}H NMR 9.56 (br s, 1H, D$_2$O-exchangeable), 9.44 (br s, 2H, D$_2$O-exchangeable), 3.36 (t, 6H, J = 6.9), 2.22 (t, 4H, J = 7.3), 2.09 (t, 2H, J = 7.4), 1.87 (s, 3H), 1.46−1.30 (m, 12H), 1.21−1.04 (m, 18H); RP-HPLC 94% (t_R = 5.01 min); LC/MS (ESI) m/z 532 (M + H)$^+$. HRMS (FAB) m/z calcd for C$_{26}$H$_{50}$N$_3$O$_8$ (M + H)$^+$ 532.3598, found 532.3595.

2,9,18,27-Tetrahydroxy-3,10,19,28-tetraoxo-2,9,18,27-tetraazanonacosane (4.2). Pale brown solid; yield 0.124 g (21%); ^{1}H NMR 9.66 (s,1H, D$_2$O-exchangeable), 9.56 (s, 1H, D$_2$O-exchangeable), 9.453 (s, 1H, D$_2$O-exchangeable), 9.446 (s, 1H, D$_2$O-exchangeable), 3.36 (t, 6H, J = 6.9), 2.98 (s, 3H), 2.22 (t, 6H, J = 7.2), 1.87 (s, 3H), 1.47−1.30 (m, 12H), 1.22−1.04 (m, 14H); RP-HPLC 82% (t_R = 4.36 min); LC/MS (ESI) m/z 533 (M + H)$^+$. HRMS (FAB) m/z calcd for C$_{25}$H$_{49}$N$_4$O$_8$ (M + H)$^+$ 533.3550, found 533.3546.

6,17,28-Trihydroxy-7,11,18,21,29-pentaoxo-6,10,17,22,-28-pentaazatriacontanamine (5.1). Colorless solid; yield 0.215 g (30%, calculated for TFA salt); ^{1}H NMR 9.73 (1H, D$_2$O-exchangeable), 9.72 (s, 1H, D$_2$O-exchangeable), 9.67 (s, 1H, D$_2$O-exchangeable), 7.81 (br t, 2H, J = 5.1, D$_2$O-exchangeable), 7.73 (br s, 3H, D$_2$O-exchangeable), 3.51−3.42 (m, 6H), 3.21 (q, 2H, J = 6.0), 3.00 (q, 2H, J = 6.0), 2.77 (br t, 2H), 2.57 (t, 2H, J = 7.4), 2.51 (t, 2H, J = 7.3, partially overlaps with DMSO-d_6 peak), 2.26 (t, 2H, J = 7.1), 2.02 (t, 2H, J = 7.5), 1.97 (s, 3H), 1.60−1.12 (m, 18H); RP-HPLC 76% (t_R = 2.93 min); LC/MS (ESI) m/z 561 (M + H)$^+$. HRMS (FAB) calcd for C$_{25}$H$_{49}$N$_6$O$_8$ (M + H)$^+$ 561.3612, found 561.3611.

7,16,27-Trihydroxy-8,17,20,28-tetraoxo-7,16,21,27-tetraazanonacosanoic Acid (5.8). Pale brown solid; yield 0.196 g (33%); ^{1}H NMR 9.57 (s, 1H, D$_2$O-exchangeable), 9.52 (s, 1H, D$_2$O-exchangeable), 7.70 (br t, 1H, D$_2$O-exchangeable), 3.36 (t, 6H, J = 6.9), 2.90 (q, 2H, J = 6.0), 2.47 (t, 2H, J = 7.2), 2.22 (t, 2H, J = 7.4), 2.17 (t, 2H, J = 7.4), 2.09 (t, 2H, J = 7.4), 1.87 (s, 3H), 1.48−1.23 (m, 12H), 1.20−1.04 (m, 10H); RP-HPLC 88% (t_R = 3.95 min); LC/MS (ESI) m/z 547 (M + H)$^+$. HRMS (FAB) m/z C$_{25}$H$_{47}$N$_4$O$_9$ (M + H)$^+$ 547.3343, found 547.3351.

7,18,29-Trihydroxy-8,12,19,22,30-pentaoxo-7,11,18,23,-29-pentaazauntriacontanoic Acid Propyl Ester (5.13). Pale brown solid; yield 0.123 g (18%); ^{1}H NMR 9.67 (s, 1H, D$_2$O-exchangeable), 9.63 (s, 1H, D$_2$O-exchangeable) 9.62 (s, 1H, D$_2$O-exchangeable), 7.79 (br t, 2H, D$_2$O-exchangeable), 3.96

(t, 2H, $J = 6.7$), 3.48−3.40 (m, 6H), 3.20 (q, 2H, $J = 5.5$ Hz), 3.00 (q, 2H, $J = 5.8$), 2.57 (t, 2H, $J = 7.2$), 2.50 (t, 2H, overlaps with DMSO-d_6 peak), 2.30−2.23 (m, 4H), 2.02 (t, 2H, $J = 7.2$), 1.96 (s, 3H), 1.63−1.33 (m, 14H), 1.28− 1.12 (m, 6H), 0.87 (t, 3H, $J = 7.4$); RP-HPLC 91% (t_R = 4.44 min); LC/MS (ESI) m/z 632 (M + H)$^+$. HRMS (FAB) m/z calcd for $C_{29}H_{54}N_5O_{10}$ (M + H)$^+$ 632.3871, found 632.3865.

3,14,25-Trihydroxy-4,8,15,18,26-pentaoxo-3,7,14,19,25-pentaazaheptacosane (5.17). Colorless solid; yield 0.171 g (31%); ^{1}H NMR 9.67 (s, 1H, D$_2$O-exchangeable), 9.63 (s, 1H, D$_2$O-exchangeable), 9.62 (s, 1H, D$_2$O-exchangeable), 7.80 (br t, 2H, D$_2$O-exchangeable), 3.51 (q, 2H, $J = 7.1$), 3.48−3.40 (m, 4H), 3.21 (q, 2H, $J = 5.8$), 3.00 (q, 2H, $J = 5.8$), 2.57 (t, 2H, $J = 7.2$), 2.49 (t, 2H, $J = 7.2$, partially overlaps with DMSO-d_6 peak), 2.26 (t, 2H, $J = 7.2$), 2.02 (t, 2H, $J = 7.2$), 1.97 (s, 3H), 1.60−1.32 (m, 8H), 1.30− 1.12 (m, 4H), 1.06 (t, 3H, $J = 7.0$); RP-HPLC 74% (t_R = 3.12 min); LC/MS (ESI) m/z 504 (M + H)$^+$. HRMS (FAB) m/z calcd for $C_{22}H_{42}N_5O_8$ (M + H)$^+$ 504.3033, found 504.3028.

4,15,26-Trihydroxy-5,9,16,19,27-pentaoxo-4,8,15,20,26-pentaazaoctacosane (5.19). Colorless solid; yield 0.170 g (30%); ^{1}H NMR 9.67 (s, 1H, D$_2$O-exchangeable), 9.62 (s, 2H, D$_2$O-exchangeable), 7.80 (br t, 2H, D$_2$O-exchangeable), 3.48−3.42 (m, 6H), 3.21 (q, 2H, $J = 6.0$), 3.00 (q, 2H, $J = 6.0$), 2.57 (t, 2H, $J = 7.6$), 2.50 (t, 2H, overlaps with DMSO-d_6 peak), 2.26 (t, 2H, $J = 7.4$), 2.02 (t, 2H, $J = 7.4$), 1.97 (s, 3H), 1.59−1.33 (m, 10H), 1.28−1.12 (m, 4H); 0.82 (t, 3H, $J = 7.4$); RP-HPLC 93% (t_R = 3.37 min); LC/MS (ES) m/z 518 (M + H)$^+$. HRMS (FAB) m/z calcd for $C_{23}H_{44}N_5O_8$ (M + H)$^+$ 518.3190, found 518.3188.

2,13,22-Trihydroxy-3,7,14,23-tetraoxo-2,6,13,22-tetraazatetracosane (7.2). Colorless solid; yield 0.121 g (25%); ^{1}H NMR 9.82 (s, 1H, D$_2$O-exchangeable), 9.66 (s, 1H, D$_2$O-exchangeable), 9.55 (s, 1H, D$_2$O-exchangeable), 7.79 (br t, 1H, D$_2$O-exchangeable), 3.45 (t, 4H, $J = 7.0$), 3.21 (q, 2H, $J = 6.7$), 3.08 (s, 3H), 2.49 (t, 2H, $J = 7.4$, partially overlaps with DMSO-d_6 peak), 2.31 (t, 2H, $J = 7.4$), 2.02 (t, 2H, $J = 7.4$), 1.96 (s, 3H), 1.57−1.40 (m, 8H), 1.30−1.12 (m, 8H); RP-HPLC 95% (t_R = 3.56 min); LC/MS (ESI) m/z 447 (M + H)$^+$. HRMS (FAB) m/z calcd for $C_{20}H_{39}N_4O_7$ (M + H)$^+$ 447.2819, found 447.2818.

3,14,23-Trihydroxy-4,8,15,24-tetraoxo-3,7,14,23-tetraazapentacosane (7.10). Colorless solid; yield 0.148 g (29%); ^{1}H NMR 9.57 (s, 1H, D$_2$O-exchangeable), 9.54 (s, 1H, D$_2$O-exchangeable), 9.45 (s, 1H, D$_2$O-exchangeable), 7.70 (br t, 1H, D$_2$O-exchangeable), 3.41 (q, 2H, $J = 7.1$), 3.36 (t, 4H, $J = 6.9$), 3.12 (q, 2H, $J = 6.4$), 2.40 (t, 2H, overlaps with DMSO-d_6 peak), 2.22 (t, 2H, $J = 7.2$), 1.93 (t, 2H, $J = 7.4$), 1.87 (s, 3H), 1.46−1.30 (m, 8H), 1.22−1.03 (m, 8H), 0.96 (t, 3H, $J = 7.0$); RP-HPLC 83% (t_R = 3.71 min); LC/MS (ESI) m/z 461 (M + H)$^+$. HRMS (FAB) m/z calcd for $C_{21}H_{41}N_4O_7$ (M + H)$^+$ 461.2975, found 461.2981.

Determination of Relative Binding Affinities for Iron. All glassware was rinsed with 0.1 N HCl and MilliQ water before use.

Preparation of 1 mM Ligand Assay Solutions. Ligand solutions were prepared from either purified or crude ligands.

In the case of purified compounds, a 5 mM stock solution in DMSO or methanol was prepared, and an aliquot (200 μL) was diluted with water (800 μL). Solutions of crude ligand in DMSO or methanol were prepared in approximate concentrations of 2−5 mM and quantitated by an internal standard method. Library compounds were divided into groups characterized by stuctural features such that the members of a given group were presumed to have equivalent molar absorptivities and λ_{max} values. From each group, a representative was selected for purification by preparative HPLC to afford a reference standard for that group. Mixtures composed of each reference standard and the internal standard (3,4-dihydroxybenzoic acid) in known concentrations were analyzed by HPLC, and response factors were determined. These response factors were applied to data from injections of crude library compounds (diluted from stock solutions) spiked with internal standard to determine the stock solution concentrations. The stock solutions were then diluted to a concentration of 1 mM with water.

Preformation of the Sulfoxine−Fe(III) Complex with Subsequent Addition of the Test Ligand. In a typical example, 60 μM sulfoxine−Fe(III) solution was formed by the addition of freshly prepared 1 mM FeCl$_3$ in 1 mM HCl (3 mL) to 10 mM sodium sulfoxine in 20 mM aqueous 1,4-piperazine-bis(ethanesulfonic acid) (PIPES, 3 mL), followed by dilution with 20 mM aqueous PIPES (pH 7) to a volume of 50 mL. Freshly prepared ligand solution (15 μL, 1 mM) was added to the sulfoxine−Fe(III) solution (250 μL), vortexed, and allowed to stand overnight (∼16 h) at room temperature. The control solution was prepared by adding water (15 μL) to the sulfoxine−Fe(III) solution (250 μL).

Preformation of the Ligand−Fe(III) Complex with Subsequent Addition of Sulfoxine. A freshly prepared 1 mM ligand solution (15 μL) was vortexed with 1 mM FeCl$_3$ in 1 mM HCl (15 μL) and left at room temperature for 15 min. After the addition with vortexing of 20 mM aqueous PIPES buffer (pH 7, 85 μL) followed by sodium sulfoxine [150 μL, 1mM (from a 10-fold dilution of 10 mM stock in PIPES)], the solution was allowed to stand at room-temperature overnight (∼16 h). The control solution was prepared by combining and vortexing 1 mM FeCl$_3$ in 1 mM HCl (15 μL), 10 mM sodium sulfoxine in PIPES (15 μL), PIPES (220 μL), and water (15 μL).

Measurements of the absorbances of the two sets of solutions described above were made at 570 nm on a microplate reader. The amount of iron stripped by the tested ligand is expressed as a percentage, $[A_0 - A_s]/[A_0] \times 100$, where A_0 is the absorbance of the initial sulfoxine−Fe complex (control), and A_s is the absorbance of the solution after addition and equilibration of uncharacterized ligand. The calculation was made for both sets of samples using the appropriate A_0. The error in the percent Fe value has been determined to be $\pm 3\%$.

References and Notes

(1) Bickel, H.; Hall, G. E.; Keller-Schierlein, W.; Prelog, V.; Vischer, E.; Wettstein, A. *Helv. Chim. Acta* **1960**, *43*, 2129−2138.

(2) Modell, B.; Berdoukas, V. In *The Clinical Approach to Thalassaemia*; Grune and Stratton: London, 1984; pp 217−241.

(3) Porter, J. B.; Huehns, E. R.; Hider, R. C. *Bailliere's Clin. Haematol.* **1989**, *2*, 257−292.

(4) Olivieri, N. F.; Brittenham, G. M. *Blood* **1997**, *89*, 739−761.

(5) Kirking, M. H. *Clin. Pharm.* **1991**, *10*, 775−783.

(6) Hershko, C.; Konijn, A. M.; Link, G. *Br. J. Haematol.* **1998**, *101*, 399−406.

(7) Kontoghiorghes, G. J.; Weinberg, E. D. *Blood Rev.* **1995**, *9*, 33−45.

(8) Hoffbrand, A. V. *Curr. Opinion Hematol.* **1995**, *2*, 153−158.

(9) Kontoghiorghes, G. J. *Toxicol. Lett.* **1995**, *80*, 1−18.

(10) Pippard, M. J.; Weatherall, D. J. *Br. J. Haematol.* **2000**, *111*, 2−5.

(11) Bergeron, R. J.; Pegram, J. J. *J. Org. Chem.* **1988**, *53*, 3131−3134.

(12) Dionis, J. B.; Jenny, H.-B.; Peter, H. H. *J. Org. Chem.* **1989**, *54*, 5623−5627.

(13) Bergeron, R. J.; Wiegand, J.; McManis, J. S.; Perumal, P. T. *J. Med. Chem.* **1991**, *34*, 3182−3187.

(14) Bergeron, R. J.; Liu, Z.-R.; McManis, J. S.; Wiegand, J. *J. Med. Chem.* **1992**, *35*, 4739−4744.

(15) Bergeron, R. J.; McManis, J. S.; Phanstiel O., IV; Vinson, J. R. T. *J. Org. Chem.* **1995**, *60*, 109−114.

(16) Roosenberg, J. M., II.; Miller, M. J. *J. Org. Chem.* **2000**, *65*, 4833−4838.

(17) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373−6374.

(18) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1997**, *119*, 2301−2302.

(19) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1998**, *120*, 2690−2691.

(20) Reichwein, J. F.; Liskamp, R. M. J. *Tetrahedron Lett.* **1998**, *39*, 1243−1246.

(21) Reddy, P. A.; Schall, O. F.; Wheatley, J. R.; Rosik, L. O.; McClurg, J. P.; Marshall, G. R.; Slomczynska, U. *Synthesis* **2001**, 1086−1092.

(22) Slomczynska, U.; Reddy, P. A.; Schall, O.; Rosik, L.; Wheatley, J. R.; Marshall, G. R. *Transfus. Sci.* **2000**, *23*, 265−266.

(23) Slomczynska, U.; Reddy, P. A.; Schall, O. F.; Osiek, T.; Naik, A.; Edwards, W. B.; Wheatley, J.; Marshall, G. R. In *Peptides: The Wave of the Future*; American Peptide Society: San Diego, 2001; pp 177−179.

(24) Marshall, G. R.; Reddy, P. A.; Shall, O. F.; Naik, A.; Beusen, D. D.; Ye, Y.; Slomczynska, U. In *Advances in Supramolecular Chemistry*; Cereberus Press: Miami, 2002; pp 174−243.

(25) Hauske, J. R.; Dorff, P. *Tetrahedron Lett.* **1995**, *36*, 1589−1592.

(26) Wisniewski, K.; Koldziejczyk, A. S.; Falkiewicz, B. *J. Pept. Sci.* **1998**, *4*, 1−14.

(27) Barlaam, B.; Koza, P.; Berriot, J. *Tetrahedron* **1999**, *55*, 7221−7232.

(28) Bauer, U.; Ho, W.-B.; Koskinen, A. M. P. *Tetrahedron Lett.* **1997**, *38*, 7233−7236.

(29) Khan, S. I.; Grinstaff, M. W. *Tetrahedron Lett.* **1998**, *39*, 8031−8034.

(30) Ede, N. J.; James, I. W.; Krywult, B. M.; Griffiths, R. M.; Eagle, S. N.; Gubbins, B.; Leitch, J. A.; Sampson, W. R.; Bray, A. M. *Lett. Pept. Sci.* **1999**, *6*, 157−163.

(31) Floyd, C. D.; Lewis, C. N.; Patel, S. R.; Whittaker, M. *Tetrahedron Lett.* **1996**, *37*, 8045−8048.

(32) Richter, L. S.; Desai, M. J. *Tetrahedron Lett.* **1997**, *38*, 321−322.

(33) Barn, D. R.; Morphy, J. R.; Rees, D. C. *Tetrahedron Lett.* **1996**, *37*, 3213−3216.

(34) Barlaam, B.; Hamon, A.; Maudet, M. *Tetrahedron Lett.* **1998**, *39*, 7865−7868.

(35) Hennequin, L. F.; Piva-Le Blanc, S. *Tetrahedron Lett.* **1999**, *40*, 3881−3884.

(36) Schwyn, B.; Neilands, J. B. *Anal. Biochem.* **1987**, *160*, 47−56.

(37) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum Press: New York, 1975.

(38) Lewandowski, K.; Murer, P.; Svec, F.; Frechet, J. M. J. *Anal. Chem.* **1998**, *70*, 1629−1638.

(39) Cavallaro, R. A.; Filocamo, L.; Galuppi, A.; Galione, A.; Brufani, M.; Genazzani, A. A. *J. Med. Chem.* **1999**, *42*, 2527−2534.

(40) Kohler, W.; Robello, D. R.; Willand, C. S.; Williams, D. J. *Macromolecules* **1991**, *24*, 4589−4599.

(41) Geyson, H. M.; Rodda, S. J.; Mason, T. J.; Tribbick, G.; Schoofs, P. G. *J. Immunol. Methods* **1987**, *102*, 259−274.