Synthesis and Biological Evaluation of Hydroxamate-Based Iron Chelators

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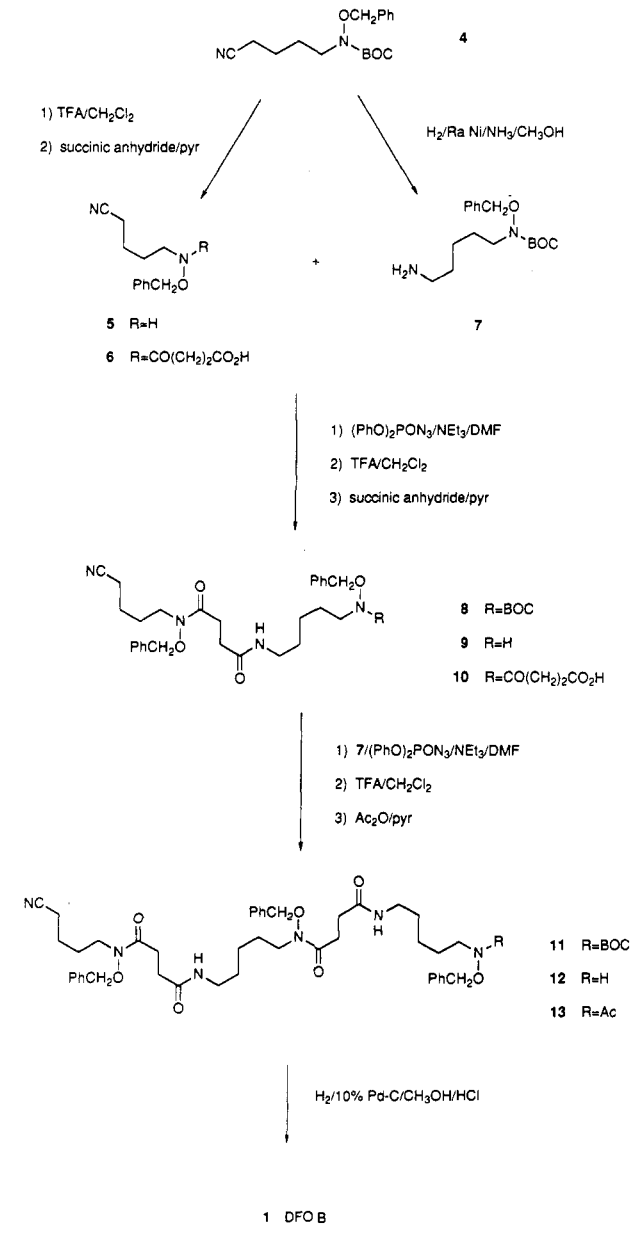
A new and versatile route to desferrioxamine B (DFO, 1) is described. Hydroxamate reagent 4 was elaborated in a series of high yield steps to the *tert*-butoxycarbonyl nitrile 11, which provided DFO in three transformations. The intermediate 11 could also be utilized in the preparation of DFO analogues which contain terminal *N*-acyl groups other than acetyl. The methodolgy was further employed in the syntheses of the DFO polyether analogues 2 and 3, beginning with the 3,6,9-trioxadecylation of *N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine. Polyethers 2 and 3 are neutral molecules, which are somewhat more lipophilic than the parent DFO. Polyether hydroxamate 2 was shown to be nearly 3 times as effective as desferrioxamine at clearing iron in rats.

Microorganisms produce a group of low molecular weight chelators, siderophores,[1-6] for the purpose of acquiring iron. The metal exists in the biosphere largely in the insoluble ferric state and would be otherwise inaccessible to bacteria without such ligands. Although a large number of siderophores have been identified, they fall largely into two structural classes: the catecholamides and the hydroxamates.[1] Many of the ligands of both structural types contain polyamine backbones. While the hexacoordinate catecholamides parabactin[2] and vibriobactin[3] are predicated on the substituted triamines spermidine and norspermidine, respectively, the hydroxamates are frequently derived from the diamines putrescine or cadaverine, or from their biochemical precursors, ornithine or lysine.[1] For example, the siderophores isolated from *Streptomyces pilosus*, desferrioxamines A-I, consist of a group of hydroxamates with either repeating putrescine or cadaverine units in their backbones.[4] The most well-known of these chelators, desferrioxamine B (DFO),[5] is a linear trihydroxamate ligand, which forms a very stable hexacoordinate, octahedral complex[6] with iron(III), $K_f = 1 \times 10^{30}$ M^{-1}. Although DFO binds a number of different 3+ cations, e.g. Al(III), Ga(III), Cr(III), it exhibits a high specificity for iron(III). It is not too surprising then that the mesylate salt of desferrioxamine, Desferal, has been employed in the treatment of several iron-overload diseases such as thalassemia.[7,8] However, the fact that patients must be continuously infused because of the drug's short half-life in the body has compelled investigators to continue the search for better therapeutic iron chelators.

Scheme I. A New and Versatile Synthesis of Desferrioxamine B (DFO, 1)



Results and Discussion

Synthesis. We report a new and versatile route to DFO (1) (Figure 1), predicated on our triprotected *N*-hydroxycadaverine reagent.[9] We have published a total synthesis

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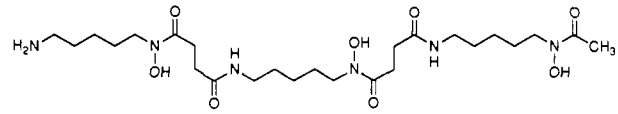
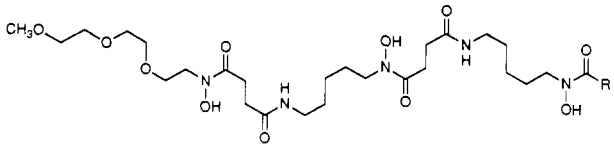


1 Desferrioxamine B (DFO)



2 R=CH_3

3 R=$CH_2O(CH_2)_2O(CH_2)_2OCH_3$

Figure 1. Desferrioxamine B (DFO, 1), acetyl triether 2, and bis(triether) 3.

of DFO,[10] in which the construction moved from the *N*-acetyl to the primary amine end of the molecule. The new scheme proceeds in the opposite direction, avoids some of the problems associated with reduction steps, is of high overall yield, and begins with easily accessible starting materials. The methodology was utilized to access several desferrioxamine analogues that showed promising activity in terms of their ability to remove iron from rats. These derivatives include polyether-containing ligands **2** and **3** (Figure 1), which were designed to be lipophilic. In both instances this change in solubility properties rendered the drug more effective than desferrioxamine at removing iron in our rodent model.

The new route (Scheme I) began with the generation and selective deprotection of triprotected *N*-hydroxycadaverine **4**, also used to prepare bisucaberin.[9] The backbone of DFO was constructed by a series of acylations such that the acetyl function was attached at the end of the synthesis. In the key synthon **4**, the primary amine was masked as a nitrile, while the hydroxylamine was *N*-(*tert*-butoxycarbonyl), *O*-benzyl diprotected. The synthesis of reagent **4** began with the conversion of *O*-benzylhydroxylamine hydrochloride to its *N*-(*tert*-butoxycarbonyl) derivative,[11] which is crystalline, stable, and available in a single step from commercial reagents. *N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine was N-alkylated with 5-chlorovaleronitrile (NaH, DMF, NaI) to give *O*-benzyl-*N*-(*tert*-butoxycarbonyl)-*N*-(4-cyanobutyl)hydroxylamine **4**. The *O*-benzyl protecting group in **4** was left intact until hydrogenation (Pd–C) to the final product **1**. *N*-(*tert*-Butoxycarbonyl)-*O*-benzylhydroxylamine can also be N-alkylated with commercially available ω-chloroalkanenitriles to either shorten or lengthen the N-hydroxydiamine chain. Thus, the length of DFO can be varied through this flexible synthetic strategy. In contrast, the previous synthesis[10] of DFO began with 4-cyanobutanal, which was made by a tedious method and is somewhat unstable. Moreover, if one wanted to vary the length of the N-hydroxydiamine chains of DFO, a new and reactive cyano aldehyde would be required.

Brief exposure of *N*-(*tert*-butoxycarbonyl) nitrile **4** to trifluoroacetic acid (TFA) resulted in collapse to carbon dioxide, isobutylene, and *O*-benzyl-*N*-(4-cyanobutyl)-hydroxylamine (**5**), thus freeing up the hydroxylamine nitrogen. Alternatively, the nitrile in **4** was selectively hydrogenated with W-2 grade Raney nickel in methanolic ammonia in the presence of the benzyl group to generate primary amine **7**. This high-yield reaction required no prewashing of the catalyst. In the previous route,[10] even with such pretreatment of the nickel catalyst, hydrogenation of nitriles which contained the base-sensitive succinate unit occurred in variable yields. Benzyloxyamine **5** was acylated with succinic anhydride in hot pyridine to give half-acid nitrile **6**.[10] If succinic anhydride were replaced with glutaric anhydride in Scheme I, homologues of DFO could easily be generated. Coupling of acid **6** with primary amine **7** was carried out with diphenyl phosphorazidate, the Yamada reagent,[12] (NEt_3/DMF) to afford masked tetracoordinate ligand **8**. The phosphoryl azide proved to be a superior amide coupling agent to dicyclohexylcarbodiimide (DCC), which had been employed in the previous synthesis.[10] The yields were consistently higher and the phosphorus-containing byproduct could be washed away from the product, in contrast to dicyclohexylurea (DCU). The elaboration was repeated: *tert*-butoxycarbonyl nitrile **8** was treated with TFA to give benzyloxyamine **9**. Acylation of **9** with succinic anhydride as before generated nitrile acid **10**, which was in turn reacted with diprotected cadaverine **7** and the Yamada reagent to give *tert*-butoxycarbonyl nitrile **11**, a versatile precursor to hexacoordinate chelators. In addition to providing DFO (**1**) in three transformations, nitrile **11** permitted us to chemically modify DFO at both the acyl and the amino termini for the purpose of determining the structure–activity relationship. The terminal *tert*-butoxycarbonyl-protecting group of **11** can be replaced with any acyl functionality that one chooses. In the previous method[10] the acetyl group was attached early in the sequence, thus restricting the versatility of that synthesis. Alternatively the cyano group in **11** could be selectively hydrogenated with prewashed Raney nickel in methanolic ammonia, and then the amino end of DFO could be modified by using an active ester or other electrophile. In order to complete the synthesis of DFO (**1**), acid-promoted deprotection of **11** to benzyloxyamine **12** was followed by acylation with acetic anhydride in pyridine to give masked DFO **13**. Simultaneous catalytic reduction of the nitrile and removal of the *O*-benzyl protecting groups as before[10] generated the natural product DFO (**1**).

The short half-life of DFO in the body and the fact that patients must be continuously infused led investigators to prepare and test analogues of DFO as potential therapeutic iron chelators. In the search for useful analogues of DFO, we previously determined that replacement of the terminal 5-aminopentyl unit of desferrioxamine B with a heptyl group rendered the molecule very insoluble in a variety of different vehicles.[10] Therefore, polyether analogues of DFO, chelators **2** and **3** in Figure 1, were prepared in order to enhance the chelator's lipophilicity as well as to maintain some degree of aqueous solubility. Specifically, in DFO polyether analogue **2**, the charged 5-aminopentyl chain was replaced with a neutral triether chain, and in

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Table I. A Comparison of the Total Iron Output Induced by DFO (po) and Analogues 2 and 3 (po), over 24 h, per Kilogram of Rat Weight

time, h	control	DFO	2	3
		Bile		
3	9.1 (±1.2)	14.7 (±5.2)	20.2 (±3.5)	16.3 (±6.5)
6	17.4 (±2.3)	43.9 (±13.7)	42.8 (±7.7)	31.7 (±7.8)
9	24.7 (±2.3)	76.8 (±19.6)	56.8 (±8.1)	42.5 (±8.0)
12	31.6 (±2.9)	97.1 (±21.9)	67.5 (±8.5)	59.7 (±14.6)
15	37.1 (±3.0)	110.8 (±22.9)	76.5 (±8.6)	67.9 (±14.8)
18	42.4 (±3.0)	119.8 (±22.9)	83.8 (±8.8)	74.6 (±14.9)
21	47.9 (±3.4)	128.7 (±23.0)	89.0 (±8.8)	81.3 (±15.2)
24	53.5 (±3.6)	135.0 (±23.1)	93.7 (±8.8)	86.6 (±15.4)
		Urine		
24	11.7 (±1.3)	16.5 (±1.0)	15.0 (±3.2)	13.3 (±6.6)
		Total		
	65.2 (±3.8)	151.5 (±23.1)	108.7 (±9.4)	99.8 (±16.7)
		Induced Iron (Treated–Control)		
	–	86.3	43.5	34.6

Table II. A Comparison of the Total Iron Output Induced by DFO (sc) and Analogues 2 and 3 (sc), over 24 h, per Kilogram of Rat Weight

time, h	control	DFO	2	3
		Bile		
3	9.1 (±1.2)	43.6 (±18.2)	117.3 (±26.7)	106.9 (±6.8)
6	17.4 (±2.3)	105.9 (±21.2)	262.5 (±27.9)	244.4 (±11.4)
9	24.7 (±2.3)	152.8 (±22.3)	374.6 (±28.7)	362.6 (±21.1)
12	31.6 (±2.9)	173.9 (+22.9)	456.1 (±32.3)	467.7 (±33.7)
15	37.1 (±3.0)	196.2 (±25.9)	519.6 (±39.2)	509.1 (±38.5)
18	42.4 (±3.0)	210.7 (±27.8)	561.2 (±42.4)	526.2 (±39.2)
21	47.9 (±3.4)	219.8 (±27.8)	585.9 (±43.4)	534.2 (±39.3)
24	53.5 (±3.6)	228.4 (±27.9)	599.5 (±43.7)	539.7 (±39.3)
		Urine		
24	11.7 (±1.3)	49.2 (±19.8)	68.4 (±17.5)	45.3 (±4.8)
		Total		
	65.2 (±3.8)	277.6 (±34.2)	667.9 (±47.0)	585.0 (±39.6)
		Induced Iron (Treated–Control)		
	–	212.4	602.7	519.8

bis(polyether) 3, the acetyl of DFO was substituted, as well, by a triether acyl group. The syntheses of chelators 2 and 3 utilized the new DFO synthetic methodology (Scheme I) and are illustrated in Scheme II.

The monomethyl ether of triethylene glycol was converted to its tosylate,[13] which was used to alkylate *N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine (NaH/DMF), resulting in polyether 14. By contrast, the methodology of the old route[10] required conversion of the alcohol to the corresponding reactive aldehyde instead of the tosylate. The alkylated product 14 was elaborated in the manner of the DFO synthesis (Scheme I): stirring it with TFA in CH_2Cl_2 gave 15, which was then acylated with succinic anhydride to afford carboxylic acid 16. Coupling of 16 and primary amine 7 was carried out in high yield with the phosphorazidate reagent to tetracoordinate equivalent 17. The protected hexacoordinate reagent 20 was obtained in high overall yield from 17 by repeating the same three conversions. Acid cleavage of the *tert*-butoxycarbonyl group in 20 furnished benzyloxyamine 21, which can be reacted with a wide range of activated esters to give any hexacoordinate polyether chelator that we choose, after catalytic debenzylation. Amine 21 was acylated with acetic anhydride (pyr/room temperature) to produce 22 or with 3,6,9-trioxadecanoyl chloride[14] (NEt_3/CH_2Cl_2) to afford 23. Catalytic debenzylation of 22 and 23 gave hexacoordinate ligands 2 and 3, respectively. Both of these chelators are soluble in water and chloroform and thus possess enhanced lipophilicity and the aqueous solubility of DFO.

Biological Results. Acetyl triether 2 and bis(triether) 3 were evaluated in a non-iron-overloaded bile duct cannulated rat model.[15,16] Studies involved both subcutaneous and oral administration of the drugs in Cremophor RH-40/water (40/60 v/v). The chelator-promoted iron excretion was monitored in both the bile and the urine, and the results were compared to those for a standard desferrioxamine dose given either orally (po) or subcutaneously (sc) also given in Cremophor RH-40/water. The rats were fasted for 48 h prior to drug administration and throughout the course of the experiment. The iron excretion data for the drugs evaluated in this study are reported in Tables I and II with comparative data for po and sc desferrioxamine. The data are presented in total "induced" iron excreted over 24 h, per kilogram of rat weight. The values are derived from the difference between the total iron excreted in test animals vs control animals on a per weight basis. This measurement does not speak to potential enterohepatic absorption, where the iron–chelator complex may be reabsorbed and thus decrease the amount of iron cleared, but only to the ability of the drug to access iron stores.

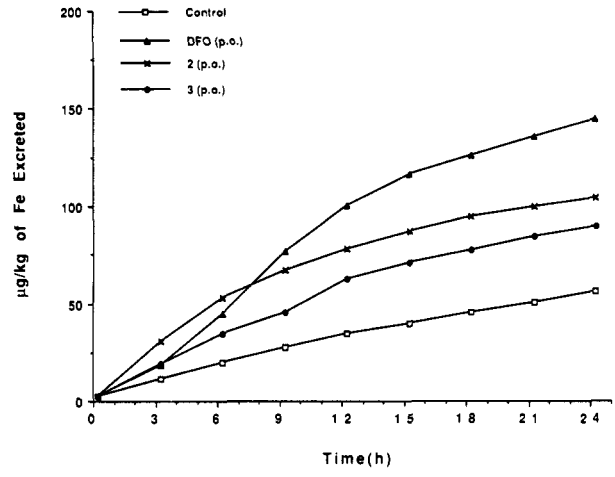

Figure 2. A comparison of the cumulative biliary iron clearance induced by analogues 2 and 3 with that of DFO when all are administered orally.

Polyether DFO analogue 2, when administered orally at 150 µmol/kg to fasted rats, was 0.50 times as effective as DFO administered orally at the same dose. Bis(triether) analogue 3, also given orally to fasted rats, was 0.40 times as effective as DFO (po) and 0.80 times as effective as chelator 2 (Figure 2). Urinary iron clearance was unremarkable in all of the animals, with iron excretion only slightly higher than that observed in the control (Cremophor/water) animals.

When either ligand 2 or 3 was administered subcutaneously at 150 µmol/kg, far more iron was excreted than with DFO administered subcutaneously at the same dose (in µmol/kg). Analogue 2 was nearly three times as effective as DFO in promoting iron clearance, while 3 was 2.5 times as effective as DFO (Figure 3).

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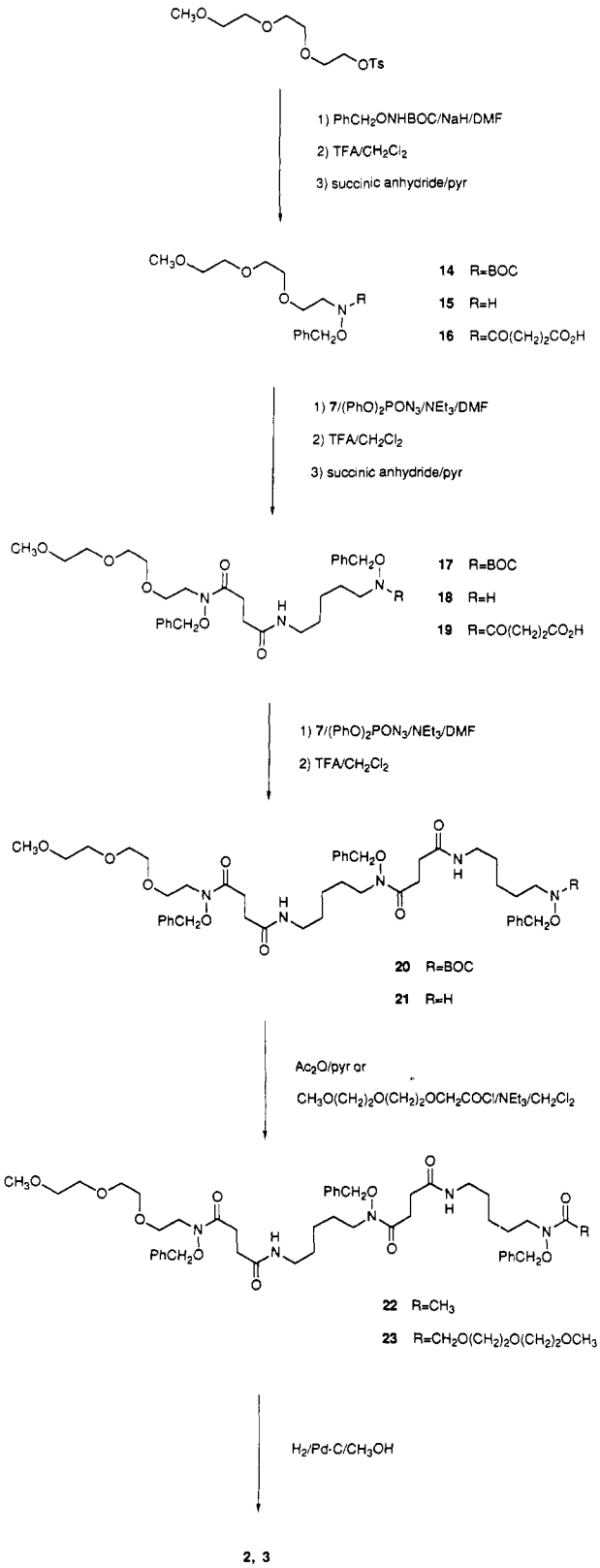
(16) Bergeron, R. J.; Wiegand, J.; Dionis, J. B.; Egli-Karmakka, M.; Frei, J.; Huxley-Tencer, A.; Peter, H. H. Evaluation of desferrithiocin and its synthetic analogues as orally effective iron chelators. *J. Med. Chem.* **1991**, *34*, 2072–2078.

Scheme II. Syntheses of DFO Polyether Analogues **2** and **3**



Biliary iron clearance induced by a single subcutaneous dose of DFO was back to baseline clearance in 15 h, with its maximum output at 6 h. Chelators **2** and **3** given subcutaneously had similar spikes at 6 h, but the iron-clearance time was protracted: levels returned to baseline only after 27 h for **2** and 21 h for **3** (Figure 4).

Experimental Section

All reagents were purchased from Aldrich Chemical Co. and were used without further purification. Fisher Optima-grade

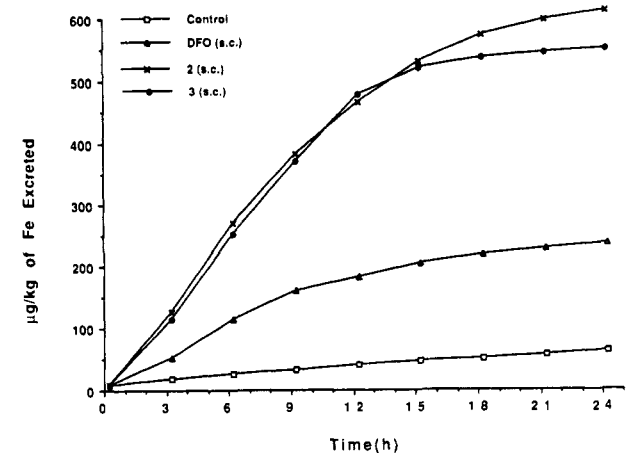


Figure 3. A comparison of the cumulative biliary iron clearance induced by analogues **2** and **3** with that of DFO when all are administered subcutaneously.

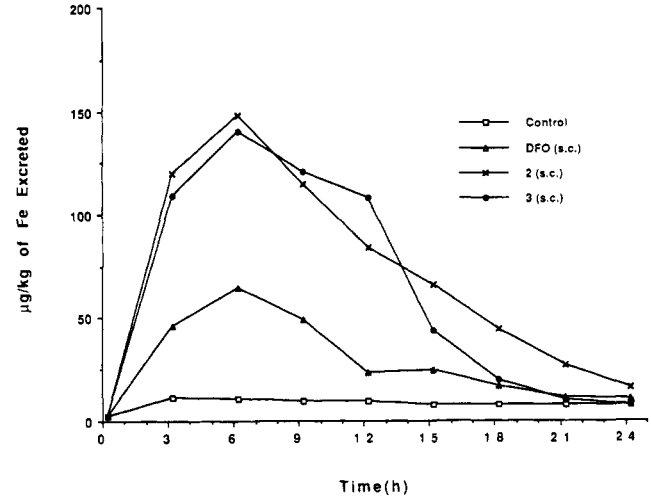


Figure 4. A comparison of the ferrokinetic clearance properties of analogues **2** and **3** with that of DFO when all are administered subcutaneously.

solvents were routinely used, and organic extracts were dried with sodium sulfate. Silica gel 60 (70–230 mesh), obtained from EM Science (Darmstadt, Germany), was used for column chromatography. Proton NMR spectra were recorded on a Varian EM-390 instrument and were run in $CDCl_3$ or D_2O with chemical shifts given in parts per million downfield from an internal tetramethylsilane or sodium 3-(trimethylsilyl)propionate standard, respectively (coupling constants are in hertz). Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. Sprague–Dawley rats were purchased from Charles River, Wilmington, MA. Bile and urine samples were analyzed on a Perkin-Elmer 5100 PC atomic absorption spectrophotometer fitted with a Model AS-51 autosampler using a quartz sampling probe as previously described.[15] Cremophor RH-40 was obtained from BASF, Parsippany, NJ.

Bile Duct Cannulation. Male Sprague–Dawley rats averaging 400 g were housed in Nalgene plastic metabolic cages during the experimental period and were given free access to water. The animals were anesthetized with sodium pentobarbital (50 mg/kg), given intraperitoneally (ip). The bile duct was cannulated with 22-gauge polyethylene tubing (Intramedic) about 1 cm from the duodenum. The cannula was inserted about 2 cm into the duct, and once bile flow was established, the cannula was tied snugly in place. A skin-tunneling needle was inserted from the shoulder area around to the abdominal incision. The cannula was threaded through the needle until it emerged from the shoulder opening.

The cannula was passed from the rat to a swivel inside a metal torque-transmitting tether, which was attached to a rodent jacket around the animal's chest. The cannula was directed from the rat to a Gilson microfraction collector by a fluid swivel mounted

above the metabolic cage. This system allowed the animal to move freely in the cage while continuous bile samples were being collected. Bile samples were collected at 3-h intervals. Urine samples were taken every 24 h.

***N*-(*tert*-Butoxycarbonyl)-*N*-(4-cyanobutyl)-*O*-benzylhydroxylamine (4)** was synthesized in these laboratories[9] in quantitative yield.

***N*-(4-Cyanobutyl)-*O*-benzylhydroxylamine (5)** was synthesized in these laboratories[9] in 81% yield.

***N*-(Benzyloxy)-*N*-(4-cyanobutyl)succinamic acid (6)** was prepared by our method in 98% yield.[9,10]

***N*-(5-Aminopentyl)-*N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine (7)** was made in 92% yield according to our published procedure.[9]

17-(*tert*-Butoxycarbonyl)-6,17-bis(benzyloxy)-7,10-dioxo-6,11,17-triazaheptadecanenitrile (8). Acid 6 (3.1 g, 10.2 mmol) and amine 7 (3.4 g, 11.0 mmol) were dissolved in distilled DMF (50 mL), and the solution was cooled to 0 °C under N_2. Diphenyl phosphorazidate (3.1 g, 11.3 mmol) and triethylamine (2.2 g, 22 mmol) were added, and the solution was stirred in the cold bath for 5 h and at room temperature for 12 h. Solvent was removed in vacuo, followed by a $CHCl_3$ workup and column chromatography on silica gel, eluting with 5% $CH_3OH/CHCl_3$, to give 6.00 g (99%) of 8.[9]

6,17-Bis(benzyloxy)-7,10-dioxo-6,11,17-triazaheptadecanenitrile (9). Trifluoroacetic acid (TFA, 40 mL) was added slowly to 8 (6.00 g, 10.1 mmol) in CH_2Cl_2 (100 mL). The solution was stirred at 0 °C for 15 min and at room temperature for 30 min. Solvents were removed by rotary evaporation, aqueous $NaHCO_3$ was added, and the product was extracted into $CHCl_3$. Column chromatography on silica gel, eluting with 5% $CH_3OH/CHCl_3$, afforded 4.3 g (87%) of 9.[9]

5,16-Bis(benzyloxy)-20-cyano-4,12,15-trioxo-5,11,16-triazaeicosanoic acid (10) was synthesized in these laboratories in 96% yield.[9]

28-(*tert*-Butoxycarbonyl)-6,17,28-tris(benzyloxy)-7,10,18,21-tetraoxo-6,11,17,22,28-pentaazaoctacosanenitrile (11). Acid 10 (3.8 g, 6.4 mmol) and amine 7 (2.2 g, 7.1 mmol) were coupled by using diphenyl phosphorazidate and the method of 8 to give, after column chromatography (5% $CH_3OH/CHCl_3$), 5.4 g (95%) of 11.[17]

6,17,28-Tris(benzyloxy)-7,10,18,21-tetraoxo-6,11,17,22,28-pentaazaoctacosanenitrile (12) was prepared by our procedure in quantitative yield.[17]

6,17,28-Tris(benzyloxy)-7,10,18,21,29-pentaoxo-6,11,17,22,28-pentaazatriacontanenitrile (13). Compound 12 (1.1 g, 1.4 mmol) was dissolved in pyridine (16 mL), acetic anhydride (4 mL) was added, and the solution was stirred for 12 h. After removal of solvents under high vacuum, water was added, followed by extraction with $CHCl_3$. The organic extracts were washed with 1 N HCl and saturated $NaHCO_3$. Column chromatography (5% $CH_3OH/CHCl_3$) gave 1.05 g (91%) of 13.[10]

Desferrioxamine B hydrochloride (1) was generated according to our published report in 84% yield.[10]

***N*-(*tert*-Butoxycarbonyl)-*N*-(3,6,9-trioxadecyl)-*O*-benzylhydroxylamine (14).** Sodium hydride (80% oil dispersion, 0.488 g, 16.3 mmol) was added to *N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine[11] (2.66 g, 11.9 mmol) in dry DMF (20 mL), and stirring was continued for several minutes. 3,6,9-Trioxadecyl tosylate[13] (4.93 g, 15.5 mmol) in DMF (3 mL) was added by syringe, and the suspension heated at 72 °C for 18 h under nitrogen. After cooling, the reaction was quenched with water (100 mL) and then extracted with ether (4 × 50 mL). The combined organic layers were washed with brine (100 mL), and solvent was removed in vacuo. Column chromatography with 4% $EtOH/CHCl_3$ produced 3.40 g (77%) of 14 as a liquid: NMR δ 1.50 (s, 9 H), 3.31 (s, 3 H), 3.5–3.7 (m, 12 H), 4.82 (s, 2 H), 7.25–7.41 (m, 5 H). Anal. ($C_{19}H_{31}NO_6$) C, H, N.

***N*-(3,6,9-Trioxadecyl)-*O*-benzylhydroxylamine (15).** Compound 14 (3.37 g, 9.12 mmol) was treated with excess TFA in CH_2Cl_2 and worked up by the method of 9 to generate 2.24 g (91%) of 15 as a liquid: NMR δ 3.07 (t, 2 H, J = 5), 3.34 (s, 3 H), 3.43–3.67 (m, 10 H), 4.67 (s, 2 H), 7.2–7.4 (m, 5 H). Anal. ($C_{14}H_{23}NO_4$) C, H, N.

***N*-(Benzyloxy)-*N*-(3,6,9-trioxadecyl)succinamic Acid (16).** A solution of 15 (2.20 g, 8.17 mmol) and succinic anhydride (1.29 g, 12.9 mmol) in pyridine (26 mL) was heated at 90 °C for 2 h under argon. After removal of the pyridine in vacuo, the residue was combined with ether (50 mL), followed by extraction with saturated aqueous $NaHCO_3$ (2 × 75 mL). The aqueous portion was extracted further with ether (2 × 50 mL), cooled to 0 °C, cautiously acidified with cold 6 N HCl (50 mL), and then extracted with $CHCl_3$ (4 × 75 mL). The $CHCl_3$ layer was washed with H_2O (50 mL), and solvent was removed by rotary evaporation. Column chromatography on silica gel (12% $CH_3OH/CHCl_3$) gave 2.79 g (92%) of 16 as an oil: NMR δ 2.52–2.73 (m, 4 H), 3.30 (s, 3 H), 3.4–3.8 (m, 12 H), 4.87 (s, 2 H), 6.53 (br s, 1 H), 7.33 (s, 5 H). Anal. ($C_{18}H_{27}NO_7$) C, H, N.

22-(*tert*-Butoxycarbonyl)-11,22-bis(benzyloxy)-12,15-dioxo-11,16,22-triaza-2,5,8-trioxadocosane (17). Acid 16 (11.0 g, 29.8 mmol) and amine 7 (9.2 g, 29.9 mmol) were coupled by using diphenyl phosphorazidate and the method of 8 to give 18.6 g (94%) of 17 as an oil: NMR δ 1.28–1.70 (m + s, 15 H), 2.42 (t, 2 H, J = 7), 3.02–3.87 (m + s, 21 H), 4.78 (s, 2 H), 4.89 (s, 2 H), 6.0 (br s, 1 H), 7.25–7.44 (m, 10 H). Anal. ($C_{35}H_{53}N_3O_9$) C, H, N.

11,22-Bis(benzyloxy)-12,15-dioxo-11,16,22-triaza-2,5,8-trioxadocosane (18). Excess TFA was added to 17 (16.0 g, 24.2 mmol) in CH_2Cl_2 at 0 °C, and the solution was stirred at room temperature and worked up by the method of 9 to give 12.0 g (87%) of 18 as an oil: NMR δ 1.24–1.62 (m, 6 H), 2.42 (t, 2 H, J = 7), 2.61–3.89 (m + s, 22 H), 4.66 (s, 2 H), 4.87 (s, 2 H), 6.0 (br s, 1 H), 7.2–7.4 (m, 10 H). Anal. ($C_{30}H_{45}N_3O_7$) C, H, N.

5,16-Bis(benzyloxy)-4,12,15-trioxo-5,11,16-triaza-19,22,25-trioxahexacosanoic Acid (19). Compound 18 (3.40 g, 6.07 mmol) was reacted with succinic anhydride in pyridine following the procedure of 16 to produce 3.81 g (95%) of 19 as an oil: NMR δ 1.2–1.8 (m, 6 H), 2.38–2.91 (m, 8 H), 3.07–3.83 (m, 19 H), 4.79 (s, 2 H), 4.88 (s, 2 H), 5.7 (br s, 1 H), 6.62 (br s, 1 H), 7.33 (s, 10 H). Anal. ($C_{34}H_{49}N_3O_{10}$) C, H, N.

33-(*tert*-Butoxycarbonyl)-11,22,23-tris(benzyloxy)-12,15,23,26-tetraoxo-11,16,22,27,33-pentaaza-2,5,8-trioxatritriacontane (20). Acid 19 (3.78 g, 5.73 mmol) and amine 7 (2.03 g, 6.58 mmol) were coupled by using diphenyl phosphorazidate and the method of 8 to give 4.22 g (78%) of 20 as a waxy solid: NMR δ 1.2–1.7 (m + s, 21 H), 2.32–2.56 (m, 4 H), 2.67–2.93 (m, 4 H), 3.05–3.88 (m, 23 H), 4.78 (s, 2 H), 4.82, (s, 2 H), 4.88 (s, 2 H), 6.23 (br s, 2 H), 7.33 (s, 15 H). Anal. ($C_{51}H_{75}N_5O_{12}$) C, H, N.

11,22,33-Tris(benzyloxy)-12,15,23,26-tetraoxo-11,16,22,27,33-pentaaza-2,5,8-trioxatritriacontane (21). Excess TFA was added to 20 (2.05 g, 2.16 mmol) in CH_2Cl_2 at 0 °C. The solution was stirred at room temperature and worked up by the method of 9 to give 1.91 g (quantitative) of 21 as a waxy solid: NMR δ 1.18–1.70 (m, 12 H), 2.32–3.33 (m, 18 H), 3.39–3.80 (m, 14 H), 4.64 (s, 2 H), 4.80 (s, 2 H), 4.87 (s, 2 H), 6.23 (br s, 2 H), 7.2–7.4 (m, 15 H). Anal. ($C_{46}H_{67}N_5O_{10}\cdot H_2O$) C, H, N.

11,22,33-Tris(benzyloxy)-12,15,23,26,34-pentaoxo-11,16,22,27,33-pentaaza-2,5,8-trioxapentatriacontane (22). Compound 21 (1.1 g, 1.29 mmol) was treated with acetic anhydride in pyridine by the method of 13 to afford 1.05 g (91%) of 22 as a waxy white solid: NMR δ 1.13–1.77 (m, 12 H), 2.06 (s, 3 H), 2.3–2.9 (m, 8 H), 2.99–3.34 (m + s, 7 H), 3.48–3.77 (m, 16 H), 4.75 (s, 2 H), 4.80, (s, 2 H), 4.87 (s, 2 H), 6.2 (br s, 2 H), 7.33 (s, 15 H). Anal. ($C_{48}H_{69}N_5O_{11}$) C, H, N.

11,22,33-Tris(benzyloxy)-12,15,23,26,34-pentaoxo-11,16,22,27,33-pentaaza-2,5,8,36,39,42-hexaoxatritetracontane (23). Compound 21 (3.91 g, 4.60 mmol) was reacted with 3,6,9-trioxadecanoyl chloride[14] (NEt_3/CH_2Cl_2) to give 4.0 g (78%) of 23: NMR δ 1.17–1.78 (m, 12 H), 2.3–2.9 (m, 8 H), 3.04–3.37 (m, 10 H), 3.4–3.8 (m, 24 H), 4.21 (s, 2 H), 4.76 (s, 2 H), 4.81 (s, 2 H), 4.88 (s, 2 H), 6.17–6.37 (m, 2 H), 7.34 (s, 15 H). Anal. ($C_{53}H_{79}N_5O_{14}$) C, H, N.

11,22,33-Trihydroxy-12,15,23,26,34-pentaoxo-11,16,22,27,33-pentaaza-2,5,8-trioxapentatriacontane (2). Compound 22 (2.6 g, 2.92 mmol) was dissolved in distilled CH_3OH (200 mL) in acid washed glassware, and 10% Pd–C (1.1 g) was introduced. The mixture was stirred under a hydrogen atmo-

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sphere for 2 h, solids were filtered using analytical grade Celite, and the filtrate was concentrated to furnish 1.56 g (86%) of **2** as an amorphous white solid: NMR (D_2O) δ 1.17–1.80 (m, 12 H), 2.11 (s, 3 H), 2.33–2.93 (m, 8 H), 3.15 (t, 4 H, J = 7), 3.33 (s, 3 H), 3.46–3.84 (m, 16 H). Anal. ($C_{27}H_{51}N_5O_{11}$) C, H, N.

11,22,33-Trihydroxy-12,15,23,26,34-pentaoxo-11,16,22,27,33-pentaaza-2,5,8,36,39,42-hexaoxatritetracontane (3). Compound **23** (2.0 g, 1.8 mmol) was debenzylated by the method of **2** to furnish 1.35 g (80%) of **3** as an amorphous solid. A sample of **3** (0.845 g) was passed through Sephadex LH-20 eluting with EtOH to afford an analytical sample (0.766 g) of **3**: NMR (D_2O) δ 1.10–1.78 (m, 12 H), 2.33–2.93 (m, 8 H), 3.15 (t, 4 H, J = 7), 3.33 (s, 6 H), 3.47–3.85 (m, 24 H), 4.38 (s, 2 H). Anal. ($C_{32}H_{61}N_5O_{14}$) C, H, N.

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Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one (TIBO) Derivatives. 2

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In the first paper of this series a new structure with anti-HIV-1 activity was disclosed and analogues were synthesized to explore the structure–activity relationship of changes in the substituent (R) attached at the N-6 position of **9**. This study describes the syntheses and anti-HIV-1 testing of analogues with variations of the five-membered urea ring of the 4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one (TIBO) structures. Although many different rings were synthesized to replace the cyclic urea of TIBO, most were found to be inactive in inhibiting the replication of the HIV-1 virus in MT-4 cells. The exceptions were replacement of the urea oxygen with sulfur or selenium to give the corresponding thio- or selenoureas. These were found to be more active than the oxygen counterparts. A small series of analogues was synthesized and tested which allowed direct comparison of urea and thiourea derivatives. Without exception, the latter were always more active than the former. The most active compound of this series (**8d**) was found to inhibit the HIV-1 virus with an IC_{50} of 0.012 μM which is comparable to that of AZT.

Introduction

In previous publications[1,2] we described the discovery of a new series of compounds, 4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one derivatives, that were assigned the acronym TIBO derivatives. The initial lead compounds, represented by structure **9**, were found to specifically inhibit replication of HIV-1 virus, the causative agent for AIDS (acquired immune deficiency syndrome). The first paper in this series[2] described variation of the N-6 substituent and the resultant structure-activity relationships (SAR). Although those compounds had specific and consistent activity against HIV-1 virus, there was a need to find compounds with a higher level of potency. The best compound from the previous paper had activity comparable to DDI (2′,3′-dideoxyinosine), which is currently undergoing clinical evaluation, but AZT (Zidovudine), the only approved drug against AIDS, was >2600 times as effective as our best analogue in blocking replication of the HIV-1 virus in the cellular assay used to determine relative potency. This publication describes efforts to systematically alter the 5-ring urea portion of the tricyclic TIBO structure **9** to determine the effects these changes might have on the relative potency of the resultant analogues to block HIV-1 replication.

Chemistry

In addition to the synthetic methods previously described[2] to obtain the basic ring system of the TIBO series, Scheme I illustrates an efficient and versatile synthesis of several analogues and intermediates. Chloroisatoic anhydride (**1**) is treated with alanine methyl ester hydrochloride in pyridine under reflux.[3] Although the *l* isomer is pictured, the scheme has been carried out with both the *d* isomer and racemic material as well. The aromatic chloro substituent in **2** acts as a convenient block to para nitration in the next step, yet can be readily removed (step 8) if desired. Treatment of **2** with cold fuming nitric acid gave a 92% yield of **3**. Reduction of **3** with lithium aluminum hydride (LAH) in refluxing glyme served to reduce both the carbonyls and the nitro functionalities to yield triamine **4**. This material was specifically monoalkylated

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