Synthesis and Evaluation of Compounds That Facilitate the Gastrointestinal Absorption of Heparin

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A family of novel compounds (delivery agents) that promote the gastrointestinal absorption of USP heparin in rats and primates has been discovered. The delivery agents in combination with heparin were administered either orally or intracolonically in an aqueous propylene glycol solution and caused dramatic increases in both plasma heparin concentrations (anti-Factor Xa) and clotting times (APTT). Using one of the most effective delivery agents in this series, an estimated relative bioavailability of 8% can be achieved following oral administration to cynomolgus monkeys. To establish a correlation between the in vivo data and an in vitro parameter, immobilized artificial membrane (IAM) chromatography was performed. Log relative *k'* values were correlated to the efficiency of oral heparin delivery.

Introduction

Parenteral administration of heparin is the preferred therapy to prevent deep vein thrombosis (DVT) and pulmonary embolism (PE) in high-risk, hospitalized patients.[1] Mechanistically, heparin exerts its anticoagulant effect by binding to antithrombin III (AT III), thereby enhancing its binding to a number of clotting factors in the intrinsic (e.g. factors IXa, XIa, VIIa) and common (e.g. thrombin, Xa) pathways. Heparin is favored over antivitamin K oral anticoagulants such as warfarin because it produces a rapid onset of anticoagulant activity and has a short physiological half-life.[2] Heparin also has a significantly lower incidence of drug−drug interactions. These pharmacological properties facilitate uncomplicated dose adjustment and contribute to heparin's relatively large margin of safety.

Clinically, heparin anticoagulation is typically monitored by its effect on clotting time using the activated partial thromboplastin time (APTT) assay. An increase in APTT to the therapeutic target range of 1.5−2.5 times baseline[2] can be obtained easily by titrating the dose and measuring APTT. Heparin concentrations in plasma can also be determined using the anti-Factor Xa assay. The therapeutic target range is 0.1−0.2 IU/mL. Continuous monitoring is generally unnecessary once a dosing schedule that achieves the target range is defined, and untoward hemmorhage rarely occurs. The major disadvantage of heparin therapy is the requirement for parenteral administration because it is ineffective when dosed orally. Thus, heparin is usually replaced by the oral anticoagulant warfarin for outpatient therapy. An oral heparin formulation would allow for continuous heparin treatment of outpatients, thereby eliminating the need to change to warfarin.

Several recent attempts to develop effective oral heparin formulations have been reported. For example, heparin complexes with hydrophobic organic bases[3,4] or spermine and lysine salts of heparin[5] have shown limited oral bioavailability upon intraduodenal administration to both rats and dogs. Simultaneous administration of heparin and organic acids has resulted in improved absorption from the small intestine of mice.[6] Other approaches include the use of oil−water emulsions,[7,8] liposomes,[9] and calcium binding molecules[10,11] to increase the absorption of orally dosed heparin. These experiments have met with marginal success.

We previously demonstrated limited absorption of heparin in rats[12] and humans,[13] evidenced by an increase in APTT, following the oral administration of microsphere-encapsulated heparin. These microspheres were prepared from either complex, uncharacterized mixtures of thermally condensed α-amino acids[14] or acylated α-amino acids, both of which undergo spontaneous molecular self-assembly to form microspheres under acidic conditions. As part of our ongoing program to improve the efficiency of oral heparin delivery, we report here our recently developed third generation of delivery agents, which are novel compounds based on non-α-amino acids. These compounds promote the oral absorption of heparin at physiological pH and offer several advantages over our earlier systems. Most importantly, these current delivery agents are well-characterized, single chemical entities, and the preparation of microsphere suspensions is no longer required to elicit enhanced absorption. Following oral administration of a solution containing a combination of heparin and a selected delivery agent to rats, monkeys, or human subjects, therapeutic levels of anticoagulation can be obtained.[13,15] Additionally, we have correlated increased oral heparin absorption to the retention time of the delivery agents on an immobilized artificial membrane (IAM) column.

Results and Discussion

Chemistry. We have prepared the N-acylated aminoalkanoic acids, compounds **1−68**, listed in Table 1 as part of our continued effort to expand our library of oral drug delivery agents[16,17] and identify delivery agents with greater potency. Previously we reported on a

Table 1. Heparin Oral Delivery Agents
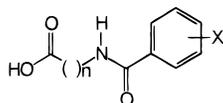
compd	n	X	log relative k'	mean peak[a] APTT (s)[b]
1	1	2-OH	−0.68	28 ± 7
2	2	2-OH	−0.63	22 ± 2
3	3	2-OH	−0.41	25 ± 2
4	4	2-OH	−0.18	26 ± 4
5	5	2-OH	0.04	24 ± 2
6	6	2-OH	0.37	56 ± 13
7	7	2-OH	0.68	125 ± 24
8	8	2-OH	1.04	163 ± 56
9	9	2-OH	1.43	153 ± 50
10	10	2-OH	1.88	51 ± 14
11	11	2-OH	2.32	44 ± 7
12	7	3-OH	0.00	23 ± 1
13	7	4-OH	−0.04	27 ± 6
14	7	H	0.09	21 ± 1
15	5	2-OCH$_3$	−0.47	27 ± 2
16	7	2-OCH$_3$	0.33	26 ± 1
17	7	2-OCF$_3$	−0.64	23 ± 1
18	7	2-F	0.11	25 ± 2
19	7	3-F	0.29	35 ± 3
20	7	4-F	0.23	31 ± 5
21	7	2-Cl	0.18	23 ± 2
22	7	4-Cl	0.65	37 ± 4
23	7	2-Br	0.24	21 ± 3
24	7	4-Br	0.76	60 ± 15
25	7	2-I	0.35	22 ± 1
26	7	3-I	1.05	39 ± 10
27	7	4-I	0.97	23 ± 1
28	7	2-CH$_3$	0.45	25 ± 2
29	7	2-NH$_2$	0.02	24 ± 1
30	7	2-COOH	−1.00[c]	23 ± 2
31	7	4-NO$_2$	0.44	33 ± 4
32	7	pentafluoro	0.41	22 ± 2
33	7	2,5-diOH	0.51	25 ± 3
34	7	2,4-diOH	0.65	37 ± 6
35	7	2,6-diOH	1.12	42 ± 9
36	7	2,3-diOH	0.46	
37	7	3,5-diOH	−0.06	20 ± 2
38	7	2-OH; 4-OCH$_3$	0.92	76 ± 16
39	5	2-OH; 4-Cl	0.26	40 ± 7
40	7	2-OH; 4-Cl	1.43	94 ± 37
41	7	2-OH; 5-Cl	0.81	56 ± 13
42	7	2-OH; 5-Br	1.54	111 ± 48
43	7	2-OH; 5-I	1.72	32 ± 6
44	7	2-OH; 4-NO$_2$	1.11	36 ± 11
45	7	2-OH; 3-CH$_3$	1.23	27 ± 2
46	7	2-OH; 4-CH$_3$	1.05	37 ± 12
47	7	2-OH; 5-CH$_3$	1.03	56 ± 15
48	7	2-OCH$_3$; 4-Cl	0.79	75 ± 15
49	7	2-OCH$_3$; 4-NO$_2$	−0.55	25 ± 2
50	7	2-Cl; 6-F	0.23	23 ± 2
51	7	2-Cl; 5-NO$_2$	0.25	23 ± 1
52	7	3-NO$_2$; 4-Cl	0.94	34 ± 4
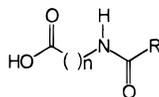
compd	n	R	log relative k'	mean peak APTT (s)
53	7	2-hydroxy-3-pyridinyl	0.90	22 ± 1
54	7	2-pyridinyl	−0.52	23 ± 2
55	7	2-thiophenyl	0.90	22 ± 2
56	7	2-pyrrolidinyl	−0.28	25 ± 4
57	7	2-chloro-3-pyridinyl	−0.53	30 ± 10
58	6	cinnamyl	0.36	39 ± 19
59	7	cinnamyl	0.70	73 ± 17
60	8	cinnamyl	0.69	140 ± 57
61	11	cinnamyl	2.29	41 ± 13
62	7	3-hydroxy-2-naphthoyl	1.70	71 ± 17

Table 1 (Continued)
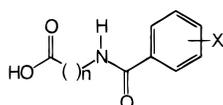
compd	n	X	log relative k'	mean peak[a] APTT (s)[b]
63	7	1-hydroxy-2-naphthoyl	2.10	31 ± 5
64	10	1-hydroxy-2-naphthoyl	>2.50	26 ± 2
65	7	cyclohexanoyl	0.15	34 ± 8
66	8	2-methoxycinnamyl	0.89	56 ± 10
67	7	(2-hydroxyphenyl)acetyl	0.12	21 ± 1
68	7	3-coumarincarbonyl	0.81	34 ± 8

[a] Mean peak APTT values occurred 30 min after dosing and remained elevated for up to 1.5 h at which time the study was ended. The baseline APTT value in rats was 21 s. [b] In vivo studies were conducted in rats by intracolonic instillation of a 25% aqueous propylene glycol solution of heparin (25 mg/kg) and a delivery agent (50 mg/kg). Data is reported as mean ± SEM. [c] This compound eluted before the void marker. The log relative k' was arbitrarily set at −1.00.
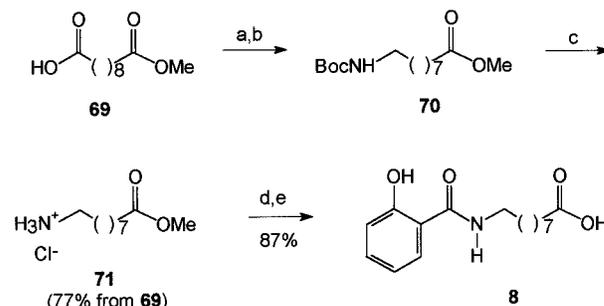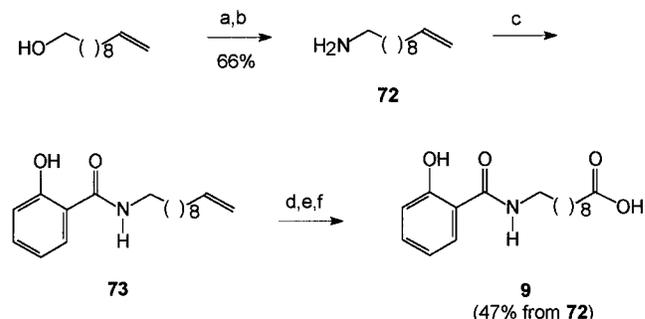
Scheme 1
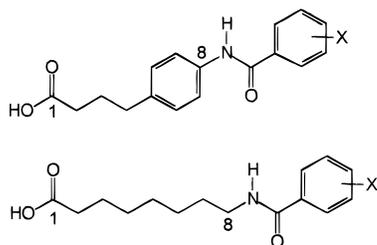
family of N-acylated amidoacids with excellent activity as oral drug delivery agents.[16] The most active compounds in this series had amide and acid functions connected by seven carbon atoms contained within an alkylaromatic framework (Scheme 1). In the current compounds, the alkylaromatic portion of the molecule is replaced by an aliphatic chain (Scheme 1). Of these materials, **7** is in clinical trials.

Compounds **1**−**7**, **10**−**28**, and **31**−**66** were prepared from the appropriate aminoalkanoic acid and acid chloride using standard techniques[18−20] under either aqueous or organic reaction conditions. In general, for aqueous reaction conditions, the aminoalkanoic acid was dissolved in aqueous base and the acid chloride was added to the solution. The mixture was stirred at room temperature for about 2 h, and the product was isolated by precipitation from the acidified reaction mixture and purified by recrystallization. Compounds **3**, **5**, **10**, **11**, **14**, **18**−**28**, **31**, **33**−**37**, **39**−**44**, **49**, **50**, **54**−**56**, **63**, and **64** were prepared by the aqueous reaction method. A nonaqueous reaction method was used to prepare **1**, **2**, **4**, **6**, **12**, **13**, **15**−**17**, **32**, **38**, **45**−**48**, **51**−**53**, **57**−**62**, **65**, and **66**. The aminoalkanoic acid was treated with chlorotrimethylsilane in dichloromethane followed by addition of triethylamine and the appropriate acid chloride.[21] The product was isolated in an aqueous workup and recrystallized. Of the compounds reported in Table 1, all were prepared by one of these two methods except compounds **8**, **9**, **29**, **30**, **67**, and **68**.

Compound **8** was prepared by the three-step reaction sequence outlined in Scheme 2. Curtius rearrangement of methyl hydrogen sebacate (**69**) gave the *t*-Boc amino ester, **70**. Crude **70** was deprotected using hydrogen chloride in dioxane to give **71** in a 77% isolated yield from **69**. Acylation of **71** with *O*-acetylsalicyloyl chloride under basic conditions gave **8** in a 67% overall yield from **69**.

Scheme 2[a]

[a] (a) DPPA, toluene, triethylamine; (b) *t*-BuOH; (c) HCl, dioxane; (d) *O*-acetylsalicyloyl chloride, triethylamine, DMF, THF; (e) 2 M NaOH.

Scheme 3[a]

[a] (a) PPh$_3$, DEAD, phthalimide, THF; (b) hydrazine, EtOH; (c) *O*-acetylsalicyloyl chloride, triethylamine, THF; (d) KMnO$_4$, Adogen 464, methylene chloride; (e) 2 M NaOH; (f) H$^+$.

Compound **9** was prepared as outlined in Scheme 3. Mitsunobu reaction of 10-undecen-1-ol followed by reaction with hydrazine gave the olefinic amine **72** in 66% yield. The amine **72** was derivatized with *O*-acetylsalicyloyl chloride, and the resulting olefin **73** was oxidized to the acid using potassium permanganate. Removal of the acetate, followed by acid precipitation, gave **9** in a 47% yield from **72**.

Compound **29** was prepared by catalytic hydrogenation of the corresponding nitro compound. Compound **30** was prepared by the reaction of 8-aminocaprylic acid and phthalic anhydride. Compound **67** was the product of a triethylamine-catalyzed ring opening of 2-coumaranone by 8-aminocaprylic acid in acetonitrile. Compound **68** was prepared by the reaction of 2-coumaranone carbonyl chloride with 8-aminocaprylic acid.

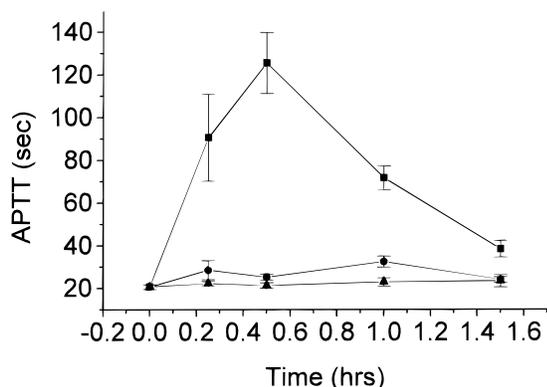**Lead Identification and Structure−Activity Re-**

Figure 1. Plasma response-time profile following intracolonic instillation of delivery agent **7** and heparin in conscious rats measured as APTT. The squares represent the response following a single, colonic dose of a combination of **7** (50 mg/kg) and heparin (25 mg/kg) in aqueous propylene glycol solution. The circles represent the response following a single, colonic dose of heparin (100 mg/kg) alone in aqueous propylene glycol. The triangles represent the response following a single, colonic dose of **7** (50 mg/kg) alone in aqueous propylene glycol. The data are plotted as mean ± SEM.

lationships. Each compound's ability to promote the gastrointestinal absorption of heparin was tested in rats. The doses of delivery agent and heparin were chosen based upon dose–response studies in which both the delivery agent and heparin were systematically varied.[25] These studies indicated that the in vivo response was dependent on the total dose of the delivery agent/heparin combination rather than the ratio of delivery agent to heparin. A dosing solution containing a combination of heparin and a delivery agent (Table 1) was instilled into the colon, and heparin absorption was measured as a change in APTT. We have previously reported that intracolonic administration is an effective screening technique for oral drug delivery.[16] Drug absorption (measured as plasma drug concentration) from the colon is comparable to drug absorption following oral gavage, but can be accomplished at lower doses of both the drug and the delivery agent.

A typical pharmacodynamic profile following intracolonic administration of heparin to rats is shown in Figure 1. The squares represent the response following a single oral dose of a 25% aqueous propylene glycol solution containing heparin (25 mg/kg) and **7** (50 mg/kg). Similar administration of control solutions containing either heparin (100 mg/kg) or **7** (50 mg/kg) alone in 25% aqueous propylene glycol are represented by the circles and the triangles, respectively. The data show that only coadministration of **7** and heparin promoted the intestinal absorption of heparin as evidenced by the 6-fold increase in APTT. Dosing of either heparin alone or **7** alone produced no dramatic elevation in APTT.

The data obtained from similar intracolonic studies conducted with delivery agents **1–68** are reported in Table 1. A number of interesting relationships between structure and activity appear evident. For instance, consider the homologous series of delivery agents **1–11**. The data show that **7**, **8**, and **9**, which have acid chain lengths of seven, eight, or nine methylene units, respectively, are the most efficient delivery agents for heparin under these dosing conditions. The compounds having either a fewer or a greater number of carbons do not effect the oral delivery of heparin as well. This is
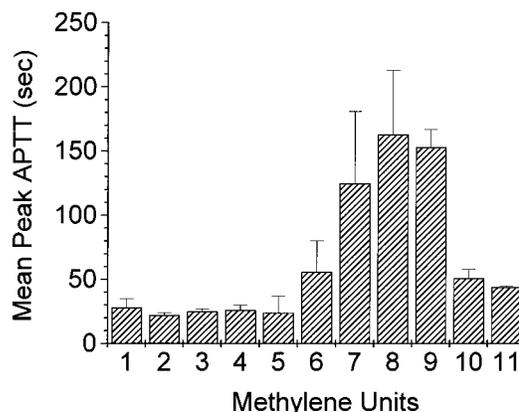

Figure 2. APTT response in rats following a single colonic dose of heparin (25 mg/kg) in combination with one of the delivery agents **1–11** (50 mg/kg) in aqueous propylene glycol solution. The data are plotted as mean ± SEM.

illustrated in Figure 2 where mean peak APTT values are plotted against the number of methylene units in the acid chain.

One possible explanation for the above observation that drug delivery activity is most efficient in combination with compounds that have a relatively long aliphatic chain is that these compounds are acting as surfactant penetration enhancers.[22–24] However, most surfactants cause damage to the gastrointestinal membranes at the concentrations required to effect drug delivery. Here, however, the increased absorption of heparin in the presence of delivery agents was not the result of frank damage to the intestinal tissue.[25] Delivery agent **7** was dosed at 300 mg/kg to rats by oral gavage or at 50 mg/kg by intracolonic instillation and animals were sacrificed at 0.5 (time of peak biological response, Figure 1), 1, and 2 h after dosing. Stomach, duodenum, jejunum, and ileum were sent to a clinical laboratory for processing and histological assessment by a DCAVP-certified pathologist. There was no evidence of pathology at any time point evaluated. Also, in preliminary studies using excised rat small intestine, we have observed increased permeability coefficients for therapeutic agents in the presence of delivery agents at concentrations that did not affect the permeation of the paracellular marker, mannitol.[26] Likewise, heparin transport across Caco-2 monoloyers is enhanced in the presence of **7** without causing damage to the cells.[27]

To demonstrate the oral delivery of heparin in a second species, **7** was tested in primates. Figure 3 shows the response following a single, oral dose of a 25% aqueous propylene glycol solution of heparin (15 or 30 mg/kg) with **7** (150 mg/kg). The squares and the circles represent the 15 and 30 mg/kg heparin doses, respectively. The data are plotted as plasma anti-Factor Xa concentration (IU/mL) versus time. The anti-Factor Xa assay measures heparin concentration in the plasma of the study animals. The bioavailability (relative to subcutaneous adminstration) of heparin following oral dosing in combination with **7** is 8.3% at a heparin dose of 15 mg/kg and a delivery agent dose of 150 mg/kg. The corresponding mean peak APTT values obtained in these studies were 80 ± 54 s (4.4 times baseline) at 150 mg/kg and 162 ± 44 s (8.9 times baseline) at 300 mg/kg. Control solutions containing either heparin alone
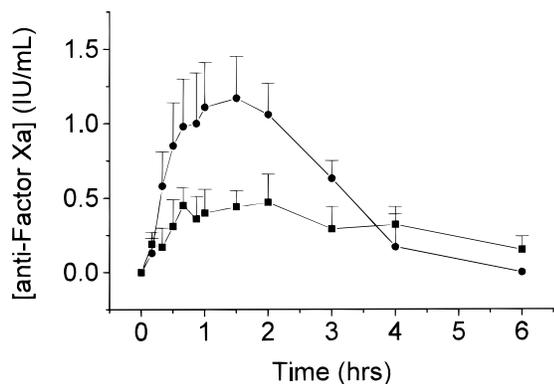
Figure 3. Dose−response study in cynomolgus monkeys at 150 mg/kg **7** and heparin doses of 15 and 30 mg/kg. The squares represent the response following a single, oral dose of **7** in combination with heparin (15 mg/kg) in aqueous propylene glycol. The circles represent the response following a single, oral dose of **7** in combination with heparin (30 mg/kg) in aqueous propylene glycol. The data are plotted as mean ± SEM.

Figure 4. APTT response in rats following a single colonic dose of heparin (25 mg/kg) in combination with one of the delivery agents **1**−**11** (50 mg/kg) in aqueous propylene glycol solution plotted as a function of log relative k' as measured by IAM chromatography. The data are plotted as mean ± SEM.

(100 mg/kg) or delivery agent alone (300 mg/kg) produced no elevations in either plasma heparin levels or APTT.

Having determined that **7** was an efficacious oral heparin delivery agent in two species, we designed 10 compounds that have different substituents at the 2-position of the aromatic ring. Thus, **14**, **16**−**18**, **21**, **23**, **25**, and **28**−**30** were prepared and tested for their ability to promote the gastrointestinal absorption of heparin. Coadministration of heparin (25 mg/kg) and each of these compounds (50 mg/kg) to rats produced only minimal increases in APTT over the baseline level, suggesting that the 2-hydroxy function was an essential structural component of the delivery agent. To test this hypothesis, we prepared **12** and **13** having the hydroxy group in the 3- and 4-positions, respectively. Both **12** (mean peak APTT 23 s) and **13** (mean peak APTT 27 s) displayed minimal activity. Thus, these data indicate that the OH group as well as its regiochemistry are critical to the drug delivery activity of this series of compounds.

On the basis of these observations, **33**−**36**, the four regioisomeric dihydroxy derivatives of **7**, were prepared and tested. Although the compounds showed activity, they were inferior in performance to **7** in the rat model (Table 1). Three hydroxy naphthoyl compounds were also prepared and tested. Interestingly, **62** showed good activity while **63** and **64** were significantly less active. Heteroaromatic amide derivatives (**53**−**57** and **68**) were also significantly less efficient oral delivery agents for heparin than their all-carbon analogues.

We next designed a group of disubstituted derivatives of **7** each having additional aromatic functionality but with the 2-OH moiety remaining constant. Of these disubstituted compounds, the most active heparin delivery agents were those having the 2-hydroxy group and an additional electronically neutral or electron-withdrawing substituent at the 5-position of the amide aromatic ring. All of these, however, were less active than **7**. Thus, coadministration of either **41** (2-hydroxy, 5-chloro), **42** (2-hydroxy, 5-bromo), or **47** (2-hydroxy, 5-methyl) and heparin produced mean peak APTT levels of 56, 111, and 56 s, respectively. Similar studies
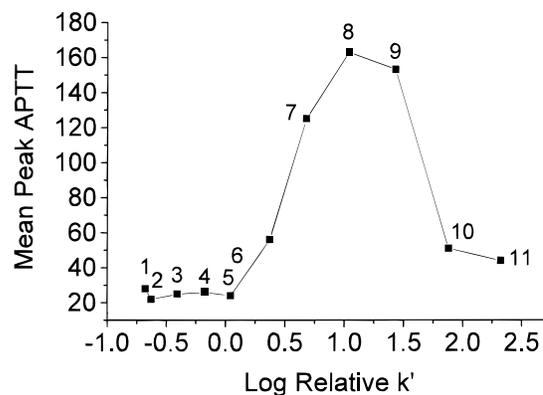
conducted with **40** and **46**, 2,4-disubstituted analogues of **41** and **47**, produced only minimal increases in APTT. Taken together, these observations indicate that the 2-hydroxybenzamide function and an aliphatic chain of seven to nine carbons are both critical for efficient heparin delivery in this series of compounds.

Chromatography Studies. We chose to study the interaction of these delivery agents with lipids to begin investigating which physical properties are important for drug delivery activity. Immobilized artificial membrane (IAM) chromatography[28] was chosen as a technique to examine these interactions because log k' has previously been correlated to partitioning into liposomes, permeability through Caco-2 cells and inverted rat intestine, and oral bioavailability in rats.[28] IAM chromatography data for compounds **1**−**11** plotted against their in vivo activity in rats are shown in Figure 4. A direct relationship was observed between aliphatic chain length and retention time, and the most active compounds (**7**, **8**, and **9**) exhibited log relative k' values ranging from 0.68 to 1.43. The remaining compounds listed in Table 1 were assayed to evaluate the importance of this lipophilicity characteristic in determining drug delivery activity, and the comparison to in vivo activity for all 68 compounds is shown in Figure 5. In this graph, the horizontal line at 30 s indicates the lower limit of activity (1.5 times baseline APTT). The most active compounds, those that had a mean peak APTT > 50 s, had a mean log relative k' of 1.04 ± 0.43, which is in the same range as **7**, **8**, and **9**. These data suggest that there is an optimal amount of interaction between the compounds and lipid molecules for maximum drug delivery activity. Those compounds having either stronger or weaker interactions are not as effective.

Conclusion

We have prepared 68 novel aliphatic acid amides that can be used as oral heparin delivery agents. These compounds can be combined with heparin in aqueous solutions and administered to rats and monkeys to effect the gastrointestinal absorption of the drug. Our studies indicate that the length of the aliphatic acid chain and the regiochemistry of the hydroxy function of the aromatic amide are critical features for determining in
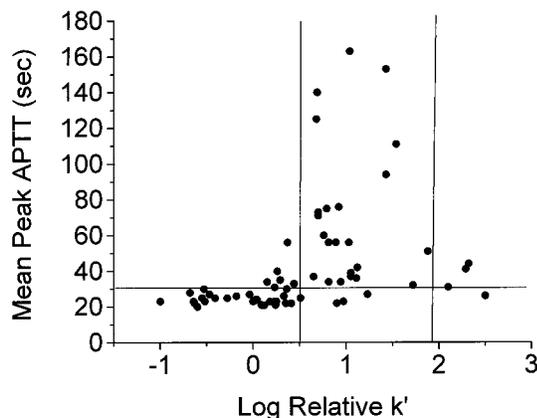
Figure 5. APTT response in rats following a single colonic dose of heparin (25 mg/kg) in combination with one of the delivery agents **1**−**68** (50 mg/kg) in aqueous propylene glycol solution plotted as a function of log relative *k*′ as measured by IAM chromatography. The data are plotted as mean ± SEM.

vivo activity of this particular group of compounds. Optimal drug delivery activity is achieved with an acid chain length of seven to nine methylene units and with a 2-hydroxybenzamide function. Chromatography studies on an IAM column have been carried out for all of these compounds, and the ability to deliver significant levels of heparin in vivo at the test doses is well correlated to their log relative *k*′ values calculated from the IAM data. This chromatography technique is now used as a preliminary in vitro screening method for new oral heparin delivery agents.

Experimental Section

Chemistry. NMR spectra were recorded at 300 MHz in either D_2O or DMSO-d_6. Combustion analyses were performed by Microlit Laboratories, Madison, NJ, and were within acceptable limits (C, H, N ± 0.4%). Thin layer chromatography (TLC) was performed using E. Merck Kieselgel 60 F-254 plates. Reactions were monitored by high-pressure liquid chromatography (HPLC) on a Vydac 25 × 4.6 mm C$_{18}$ Protein and Peptide column using a 0−50% gradient of acetonitrile in water with 0.1% trifluoroacetic acid. Melting points were performed using a Mel-Temp II from Laboratory Devices. All chemicals used in the syntheses of compounds **1**−**68** were purchased from Aldrich Chemical Co., St. Louis, MO.

General Procedures for the Preparation of Delivery Agents. With the exception of **8**, **9**, **29**, **30**, **67**, and **68**, all of the compounds in Table 1 were prepared by either method A or method B. The preparation of **7** is given as a representative example.

Method A. 8-(2-Hydroxybenzoyl)aminooctanoic acid (7). A solution of 8-aminocaprylic acid (100.0 g, 650 mmol) in aqueous sodium hydroxide (2 M, 1.4 L) was placed in a 3 L three-neck round-bottom flask fitted with an overhead mechanical stirrer, a thermometer, and an ice/water bath. *O*-Acetylsalicyloyl chloride (198.6 g, 760 mmol) was added portionwise over about 7 h while maintaining the reaction temperature at about 5 °C. The mixture was stirred at about 5 °C for 12 h. The resulting yellow solution was adjusted to about pH 6.5 with hydrochloric acid (1 M) and extracted with ethyl acetate (2 × 600 mL). The combined organic layers were dried over anhydrous sodium sulfate and evaporated under vacuum. The residue was dissolved in a minimal volume of 2 M aqueous sodium hydroxide. This solution was adjusted to about pH 6.5 with stirring, and a solid formed. The solid was collected by filtration, washed with water (3 × 300 mL), and recrystallized from 55% v/v aqueous methanol to give **7** as an off-white solid (99.7 g, 57%), mp 116−117 °C. ¹H NMR (300

MHz, DMSO-d_6): δ 8.80 (1H, s), 7.85 (1H, m), 7.45 (1H, m), 6.85 (2H, m), 3.25 (2H, m), 2.20 (2H, t), 1.50 (4H, m), 1.35 (8H, br m).

Compounds **3**, **5**, **6**, **10**, **11**, **14**, **18**−**28**, **31**, **33**−**37**, **39**−**44**, **49**, **50**, **54**−**56**, **63**, and **64** were prepared by this method.

Method B. 8-(2-Hydroxybenzoyl)aminooctanoic Acid (7). 8-Aminocaprylic acid (44.5 g, 280 mmol) in dichloromethane (560 mL) was placed in a 2 L round-bottom flask equipped with a magnetic stirrer and a reflux condenser. Chlorotrimethylsilane (62.36 g, 570 mmol) was added to the resulting suspension in one portion, and the mixture was heated to reflux for 1 h under argon. The reaction was allowed to come to room temperature and was then cooled to <10 °C in an ice/water bath. The reflux condenser was replaced with an addition funnel, and triethylamine (42.5 g, 420 mmol) was added dropwise over 15 min. A white solid formed during the course of the addition. The funnel was replaced by another addition funnel, and *O*-acetylsalicyloyl chloride (55.60 g, 280 mmol) in dichloromethane (100 mL) was added dropwise over 30 min. The reaction mixture was stirred in the ice/water bath for another 30 min and at ambient temperature for 1 h. The dichloromethane was removed by evaporation under vacuum to give a brown oil. The brown oil was cooled in an ice/water bath, and an ice-cold solution of aqueous sodium hydroxide (2 N, 700 mL) was added. The ice bath was removed, and the reaction was stirred for 2 h to give a clear brown solution. The solution was acidified with sulfuric acid (2 M, 700 mL) and stored at 5 °C for 1 h. A yellow solid formed and was collected by filtration. The solid was washed with water (3 × 100 mL) and recrystallized from 50% v/v aqueous ethanol to give **7** as tan needles (65.7 g 78%).

Compounds **1**, **4**, **6**, **12**, **13**, **15**−**17**, **32**, **38**, **45**, **51**−**53**, **57**−**62**, and **6** were prepared by this method.

Methyl 9-(*tert*-Butoxycarbonylamino)nonanonate (70). Diphenylphosphoryl azide (46.76 g, 170 mmol) was added to a mixture of methyl hydrogen sebacate (35.00 g, 162 mmol), triethylamine (17.19 g, 170 mmol), and toluene (200 mL). The reaction was heated at 80 °C for 2 h and allowed to cool to room temperature. Dry *tert*-butyl alcohol (100 mL distilled from calcium hydride) was added, and the solution was heated to reflux for 12 h. The toluene and excess *tert*-butyl alcohol were evaporated under vacuum, and ether (300 mL) was added. The resultant solution was filtered through silica. The silica was washed with ether (300 mL), and the combined ether solutions were evaporated under vacuum to give methyl **70** as a colorless oil. The oil contained traces of toluene but was used without further purification. ¹H NMR (300 MHz, DMSO-d_6): δ 6.72 (1H, m), 3.56 (3H, s), 2.87 (2H, q), 2.26 (2H, t), 1.60−1.10 (21H, three overlapping signals, m, s, bs).

Methyl 9-Aminononanonate Hydrochloride (71). Methyl 9-(*tert*-butoxycarbonylamino)nonanonate (**70**) obtained above was dissolved in dioxane (80 mL). A 4 M HCl solution in dioxane (80 mL) was added at a rate to control bubbling, and the mixture was stirred at room temperature for 4 h. A white precipitate formed and was collected by filtration through a scintered glass funnel. The white solid was washed with dioxane (100 mL) and ethyl acetate (150 mL) to give **71** (27.88 g, 77% from methyl hydrogen sebacate). ¹H NMR (300 MHz, DMSO-d_6): δ 8.11 (3H, bs), 3.55 (3H, s), 2.70 (2H, t), 2.26 (2H, t), 1.51 (4H, m), 1.23 (8H, bs).

9-(2-Hydroxybenzoyl)aminononanoic Acid (8). A solution of *O*-acetylsalicyloyl chloride (24.68 g, 124 mmol) in THF (300 mL) was cooled in an ice/water bath. Triethylamine (25 g, 249 mmol) was added dropwise, followed by the dropwise addition of a solution of **71** (27.88 g) in DMF (190 mL). The reaction mixture was stirred in the ice/water bath for 20 min and at room temperature for 2 h. The THF was removed by evaporation under vacuum to give a pink solution. The pink solution was cooled in an ice/water bath, and 2 M sodium hydroxide (300 mL) was added. The resultant solution was stirred at room temperature for 12 h and then acidified with 2 M hydrochloric acid (500 mL). Upon cooling, a solid was formed and was collected by filtration. Subsequent recrystallization from 50% v/v ethanol/water gave **8** as an off-white

solid, mp 94−96 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.73 (1H, s), 11.95 (1H, s), 8.80 (1H, t), 7.83 (1H, m), 7.37 (1H, m), 6.86 (2H, m), 3.26 (2H, m), 2.17 (2H, t), 1.49 (4H, m), 1.26 (8H, br m).

1-Aminoundec-10-ene (72). A mixture of 10-undecene-1-ol (25.00 g, 147 mmol), triphenylphosphine (38.52 g, 147 mmol), and phthalimide (21.64 g, 147 mmol) in dry THF (150 mL) was stirred vigorously under argon. The reaction mixture was then placed in an ice/water bath. Diisopropyl azodicarboxylate (DIAD, 29.71 g, 147 mmol) was dissolved in THF (50 mL) and added dropwise via an addition funnel. Upon completion of the addition, the reaction was stirred at room temperature for 4 h. The solvent was evaporated under vacuum, and ether (150 mL) was added to precipitate the triphenylphosphine oxide and hydrazine dicarboxylate which were removed by filtration. The precipitate was rinsed with ether (2 × 150 mL), and the combined filtrates were evaporated to give a yellow solid. The solid was triturated with warm hexanes (3 × 200 mL) and filtered. The combined hexanes were evaporated to give 1-phthalimidylunden-10-ene as a yellow wax.

The yellow wax was dissolved in ethanol (200 mL). Hydrazine monohydrate (7.35 g, 147 mmol) was added. The mixture was refluxed for 2 h to give a suspension. After the mixture was cooled to room temperature, concentrated hydrochloric acid (150 mL) was added, and the solid was filtered through a sintered glass filter. The residue was washed with water (150 mL), and the combined filtrates were evaporated to give a yellow solid. The solid was dissolved in sodium hydroxide (1 M, 250 mL) and extracted with ether (2 × 200 mL). The ether was dried and evaporated to give a yellow oil. The oil was purified by vacuum distillation (0.1 mmHg, 49−55 °C) to give **72** as a clear, colorless oil (15.52 g, 62.5%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.77 (1H, br m). 4.94 (2H, m), 2.48 (2H, m), 1.99 (2H, m), 1.23 (14 H, br m).

1-[O-Acetyl(2-hydroxybenzoyl)amino]undec-10-ene (73). O-Acetylsalicyloyl chloride (18.23 g, 91.8 mmol) in THF (150 mL) was cooled in an ice/water bath. Triethylamine (9.28 g 91.8 mmol), followed by 1-aminoundec-10-ene (15.52 g, 91.8 mmol) in THF (50 mL), was added dropwise via an addition funnel. The ice/water bath was removed, and the reaction was stirred overnight at room temperature. The solvent was removed by evaporation under vacuum, and the residue was dissolved in ethyl acetate (200 mL). The ethyl acetate was washed with water (2 × 150 mL). The organic layer was dried with sodium sulfate, decolorized with activated charcoal, and evaporated to give **73** as a tan solid (29.85 g, 98%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.24 (1H, t), 7.50 (2H, m), 7.30 (1H, m), 7.15 (1H, m), 5.78 (1H, m), 4.95 (2H, m), 3.15 (2H, q), 2.19 (3H, s), 1.99 (2H, m), 1.47 (2H, m), 1.26 (12H, br s).

10-(2-Hydroxybenzoyl)aminodecanoic Acid (9). Potassium permanganate (42.74 g, 270 mmol) was added to a mixture of sulfuric acid (9 M, 60 mL) and glacial acetic acid (30 mL) in water (1500 mL). The mixture was stirred rapidly with cooling in an ice/water bath. A solution of **73** (29.85 g, 90 mmol) and Adogen 464 (1.65 g) in methylene chloride (500 mL) was added dropwise over 45 min. After the addition was complete, the reaction mixture was allowed to come to room temperature and stirred for 5 h. Sodium bisulfite (40 g) was added to decolorize the reaction mixture. The organic layer was separated, and the aqueous layer was washed with methylene chloride (3 × 200 mL). The combined methylene chloride layers were dried over sodium sulfate and concentrated under vacuum. The resulting tan solid was dissolved in 50% v/v aqueous methanol, decolorized with activated carbon, and recrystallized. Upon cooling, a white solid precipitated and was isolated by filtration. After being dried overnight in a vacuum oven, **9** was obtained as a tan solid (13.07 g, 45.5%), mp 85−87 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.72 (1H, s), 11.95 (1H, s), 8.80 (1H, t), 7.82 (1H, m), 7.37 (1H, m), 6.86 (2H, m), 3.27 (2H, m), 2.17 (2H, t), 1.51 (4H, m), 1.24 (10H, br m).

8-(2-Aminobenzoyl)aminooctanoic Acid (29). 8-(2-Nitrobenzoyl)aminooctanoic acid (1.5 g, 5 mmol) was dissolved

in methanol (100 mL), and 10% palladium on carbon (0.5 g) was added. The reaction was stirred overnight at room temperature under an atmosphere of hydrogen. The resulting suspension was filtered in an inert amosphere, and the methanolic filtrate was evaporated under vacuum to give **29** as a tan solid (1.0 g, 73.9%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.15 (1H, t), 7.42 (1H, d), 7.1 (1H, t), 6.65 (1H, d), 6.5 (1H, t), 6.35 (2H, s), 3.2 (2H, q), 2.1 (2H, q), 1.5 (4H, m), 1.25 (6H, m).

8-(2-Carboxybenzoyl)aminooctanoic Acid (30). 8-Aminocaprylic acid (5 g, 0.31 mmol) was dissolved in water (50 mL). A solution of phthalic anhydride (4.64 g, 0.31 mmol) in dioxane (20 mL) was added rapidly via an addition funnel, and the mixture was stirred overnight at room temperature. The pH of the reaction mixture was adjusted to ∼1 with concentrated hydrochloric acid, and the mixture was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried over magnesium sulfate and concentrated under vacuum. The residue was recrystallized from ethyl acetate to give **30** as a white solid (2.45 g, 25.4%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.5 (1H, s), 8.25 (1H, t), 7.7 (1H, dd), 7.5 (2H, m), 7.4 (1H, dd), 3.2 (2H, q), 2.2 (2H, t), 1.5 (4H, m), 1.3 (6H, m).

8-[(2-Hydroxyphenyl)acetyl]aminocaprylic Acid (67). 2-Coumaranone (4.21 g, 31.4 mmol) was dissolved in acetonitrile (75 mL) by stirring under an argon atmosphere. Triethylamine (3.18 g, 31.4 mmol) and 8-aminocaprylic acid (5.00 g, 31.4 mmol) were added, a tan slurry formed, and the reaction mixture was heated at reflux overnight. The reaction was cooled to room temperature and concentrated under vacuum. The residue was dissolved in methylene chloride, and the organic phase was washed with aqueous hydrochloric acid (2 N, 2 × 100 mL). The organic phase was then dried over anhydrous sodium sulfate and concentrated under vacuum. The resulting tan solid was dried overnight to give **67** as a tan solid (8.35 g, 70.4%), mp 93−7 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.81 (1H, s), 8.66 (1H, t), 7.94 (1H, dd), 7.72 (1H, td), 7.48 (1H, d), 7.41 (1H, td), 3.29 (2H, q), 2.17 (2H, t), 1.47 (4H, m), 1.27 (6H, m).

8-[N-(3-Coumarincarbonyl)]aminocaprylic Acid (68). A mixture of salicylaldehyde (8.8 mL, 82.7 mmol), dimethylmalonate (13.8 mL, 90 mmol), piperidine (1.1 mL), and acetic acid (0.45 mL) in ethanol (50 mL) was heated to reflux for 3 h. Hot water (50 mL) was added to the reaction, and the resulting mixture was cooled to room temperature. A white solid formed. The reaction mixture was further cooled in a ice/water bath for 1 h prior to filtration. The white solid was collected by filtration, and the isolated solid was recrystallized from 40% aqueous ethanol v/v to give 3-(methoxycarbonyl)-coumarin as white needles (11.0 g, 61%), mp 111−113 °C. ^{1}H NMR (CDCl$_3$): δ 8.58 (1H, s), 7.52−7.88 (2H, m), 7.31−7.42 (2H, m), 3.95 (3H, s).

Aqueous hydrochloride acid (3 N, 200 mL) was added portionwise to a hot mixture of 3-(methoxycarbonyl)coumarin (5.0 g, 22.9 mmol) in ethanol (100 mL). The resulting solution was heated at 55−60 °C for 2 h. A white solid formed, and the mixture was cooled to room temperature. The solid was filtered and recrystallized from ethanol to give 3-coumarin-carboxylic acid as a white crystalline solid (3.38 g, 78%), mp 187−190 °C. ^{1}H NMR (CDCl$_3$): δ 12.25 (1H, br s), 8.97 (1H, s), 7.75−7.92 (2H, m), 7.75 (2H, d).

A mixture of 3-coumarincarboxylic acid (1.9 g, 10.0 mmol) and thionyl chloride (20 mL) was heated to reflux for 3 h. The excess thionyl chloride was evaporated under vacuum, and toluene (5 mL) was added to the residual yellow solid. The toluene was evaporated under vacuum, and the product was used without further purification.

Aqueous sodium hydroxide (5%, 14 mL, 21.5 mmol) was added to a mixture of 8-aminocaprylic acid (1.62 g, 10.15 mmol) in methylene chloride (20 mL). The mixture was cooled in an ice/water bath, and the acid chloride from the preceding step was added in a methylene chloride/THF solution (2:5, 28 mL). The mixture was stirred at 0 °C for 2 h, allowed to come to room temperature, and stirred for an additional 2 h. The solvent was evaporated under vacuum, and the residue was

acidified with hydrochloric acid (3 N). The solid formed was collected by filtration and recrystallized from aqueous methanol to give **68** as a white solid (2.57 g, 77%), mp 93−97 °C. ^{1}H NMR (DMSO-d_6): δ 8.81 (1H, s), 8.66 (1H, t), 7.95 (1H, dd), 7.72 (1H, dd), 7.38−7.50 (2H, m), 3.29 (2H, t), 2.17 (2H, t), 1.40−1.57 (4H, m), 1.20−1.30 (6H, m).

Immobilized Artificial Membrane (IAM) Chromatography. High-pressure liquid chromatography was carried out on a 30 × 4.6 mm IAM.PC.C$_3$/C$_{10}$ column (Regis Technologies, Inc.) at ambient temperature with an Hitachi L-6200A pump, an Hitachi AS-2000 autosampler, and a Perkin-Elmer PE−95 UV/vis detector. Data were analyzed using Turbochrom 4 software. The mobile phase was 10 mM sodium phosphate, pH 6.4, in 25% aqueous propylene glycol. A flow rate of 2 mL/min was used. Compounds for analysis were prepared at 100 μg/mL in mobile phase; the injection volume was 50 μL, and detection of compounds was conducted at 220 nm. Potassium iodide was employed to mark the void time of the column. Capacity factor values (K') were calculated according to the equation:

$$K' = (t_R - t_0)/t_0$$

where t_R is the retention time of the analyte and t_0 is the void time of the column.

Two compounds were included in each chromatography run as standards. 4-(Phenylsulfonyl)aminobenzoic acid (**74**)[16] was included before and after each set of five compounds being analyzed. A mean K' value was calculated from the data for the two injections of **74** that bracketed the test set of five compounds, and this mean K' value was used to calculate a relative K' value for each compound (K' value for compound X/K' value for **74**). The data were converted to log relative K' values when comparison was made to in vivo results. 4-[4-(2-Hydroxybenzoyl)amino]phenylbutyric acid (**75**)[16] was included at the beginning and at the end of each chromatography run to check the performance of the column and HPLC system performance; the log relative K' value for each injection of **75** was expected to fall within the range 1.10−1.14.

Animal Experiments. All animal experimental procedures and protocols were approved in advance by the Emisphere and New York Medical College or ITR Laboratories Canada Institutional Animal Care and Use Committees. Plasma samples were harvested from citrated (2.7%) whole blood using a Beckman microfuge. The APTT assay[29] was performed using a BBL fibrometer (VWR Scientific, South Plainfield, NJ), and APTT reagents were purchased from Sigma Diagnostics, St. Louis, MO. The anti-Factor Xa assay was performed using a kit from Chromogenix, MoIndal, Sweden. Heparin (164 IU/mg) was purchased from Scientific Protein Laboratory, Waunakee, WI. Ketamine hydrochloride was purchased from Parke Davis, Holland, MI. Pharmaceutical grade propylene glycol was purchased from Sigma Chemical Company, St. Louis, MO.

Dosing Solution Preparation for Rat Studies. The delivery agent (150 mg) and heparin (75 mg) were mixed by vortex as dry powders. This dry mixture was dissolved in 25% v/v aqueous propylene glycol, and the apparent pH was adjusted to about 7 with 2 N aqueous sodium hydroxide. The final volume was adjusted to 3.0 mL. The dosing solution was sonicated to produce a clear, colorless solution with an apparent pH of about 7.

Rat Studies. Male Sprague−Dawley rats, housed in the animal facility at New York Medical College, Valhalla, NY, were acclimated for a period of at least 5 days prior to dosing. The animals weighed 300−350 g and were fasted for 12 h before dosing. Groups of five or six rats were anesthetized with 44 mg/kg ketamine hydrochloride intramuscularly (im) immediately prior to dosing. Each group was administered a single colonic dose (1 mL/kg) of either the delivery agent/heparin combination or the control solution via a 7.5 cm, 8 fr. Rusch catheter attached to a 1 mL syringe. The dosing catheter was inserted into the colon through the anus until the tube was no longer visible, and the dosing solution was expressed slowly into the colon. Citrated blood samples were collected serially by cardiac puncture at 0, 0.25, 0.5, 1.0, and 1.5 h, plasma harvested, and APTT measured.

Dosing Solution Preparation for Primate Studies. The total volume of dosing solution required for the study was predetermined from a standard dosing volume of 3 mL/kg. The required amount of **7** or **9** and USP heparin as a dry mix were dissolved in 25% v/v aqueous propylene glycol. This solution was sonicated and heated at 37 °C for 20−30 min. The pH of the resulting solution was 7.0−8.0.

Non-Human Primate Studies. The studies were conducted at ITR Laboratories Canada, Inc. A group of four cynomolgus monkeys, two males and two females, weighing 2−3 kg were fasted for 4 h prior to dosing and up to 2 h postdosing. The animals were anesthetized with an intramuscular dose of 10 mg/kg ketamine hydrochloride immediately prior to dosing. Each animal was administered 3 mL/kg of the dosing solution via a nasogastric tube. Citrated blood samples (1 mL each) were collected by venipuncture at 1 h predosing and at 10, 20, 30, 40, and 50 min and 1, 1.5, 2, 3, 4, and 6 h postdosing. The harvested plasma was divided into two aliquots. One was used immediately for APTT analysis, and the other was frozen at −80 °C and shipped to Emisphere for anti-Factor Xa assay.[30]

Data Analysis. Descriptive statistics (means and standard errors of the mean) were calculated using the Origin software package version 3.5, and the mean APTT for each test combination was plotted versus time.

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