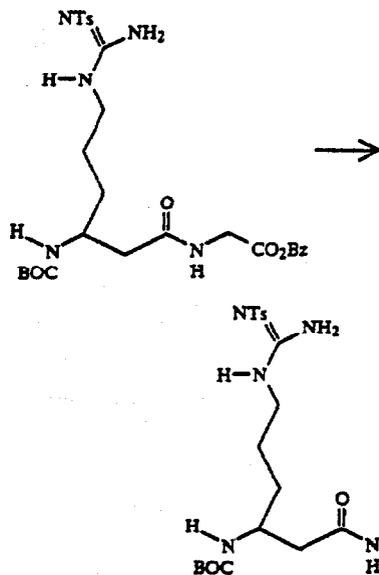
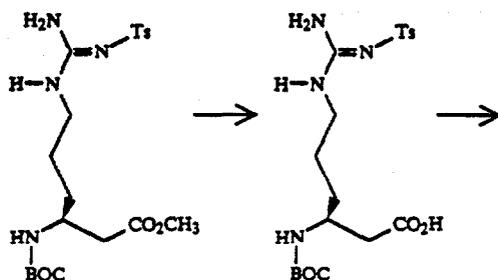
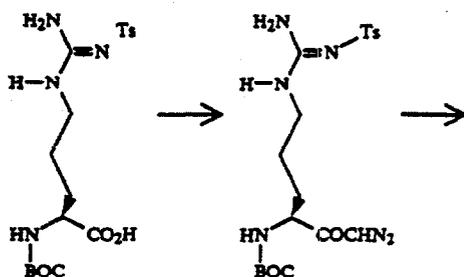


27



N $^{\alpha}$ -BOC-N $_{\epsilon}$ -Tos-AroinineDiazomethylketone

We stirred 10 g (13.4 mmole) of N $^{\alpha}$ -BOC-N $_{\epsilon}$ -Tos-arginine (Bachem, Torrance, CA) and 2.1 ml (19.1 mmole) of N-methylmorpholine (Aldrich, Milwaukee, Wis.) in 100 ml anhydrous tetrahydrofuran (THF) under argon for 5 minutes at room temperature. The solution was then cooled to -15° C. and 2.8 ml (21.6 mmol) of isobutylchloroformate (Aldrich) was added. We continued to stir the reaction mixture at -15° C. for 5 minutes, and then filtered it through a pad of Celite/MgSO $_4$. We next added the filtrate to an ice-cold ethereal solution of diazomethane (150 mM, generated from 32.4 g Diazald; Aldrich). The solution was stirred and allowed to gradually reach ambient temperature overnight. The solvent was then removed in vacuo and the residue dissolved in 200 ml chloroform. We then washed the organic solution successively with 200 ml of saturated NaHCO $_3$, followed by 200 ml of saturated NaCl, dried it over anhydrous MgSO $_4$, and concentrated it again to an oily residue. The residue was then

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purified by flash chromatography on a 4 \times 17 cm column of silica gel using a step gradient of acetone in chloroform (10% acetone in 2 l chloroform, followed by 20% acetone in 3 l chloroform). Fractions of 25 ml were collected. Aliquots of each fraction were assayed by thin-layer chromatography (TLC). Fractions containing the desired product were pooled and evaporated to dryness. The product, diazomethylketone, was purified as a pale yellow foam (6.54 g).

N $^{\alpha}$ -BOC-N $_{\epsilon}$ -Tos- β -Homoarginine Methyl ester

We dissolved the diazomethylketone prepared above in 100 ml of anhydrous methanol and refluxed that solution under argon while a solution of silver benzoate catalyst (165 mg in 400 μ l triethylamine) was added dropwise. After 30 minutes, the refluxing solution was cooled to room temperature, slurried with Norit, and filtered through Celite. The solvent was then removed in vacuo and the oily residue purified by flash chromatography over silica gel. Elution was achieved with 4 l of 10% acetone in chloroform. The desired product, β -homoarginine methyl ester, was thus purified as a light tan foam (6.43 g).

N $^{\alpha}$ -BOC-N $_{\epsilon}$ -Tos- β -Homoarginine

We dissolved all of the above methyl ester in 100 ml of methanol and then reacted it with a solution of LiOH (148 g in 50 ml water) overnight at room temperature under argon with constant stirring. We removed the methanol in vacuo, dissolved the residue in water and washed it with ethyl acetate. We next added saturated citric acid until the solution reached a pH of 4. We then extracted the resulting carboxylic acid into ethyl acetate. The extraction was repeated at pH 3, and the combined organic phases were dried over MgSO $_4$ and concentrated in vacuo. The resulting crude acid was recovered as a white foam (4.9 g). The acid was further purified on a Vydac C $_{18}$ reverse-phase HPLC column, as described in Example 4, except that the effluent stream was monitored at 214 nm. Following lyophilization of the desired fractions, the product, N $^{\alpha}$ -BOC-N $_{\epsilon}$ -Tos- β -homoarginine, was recovered as a white amorphous solid.

A sample of the N $^{\alpha}$ -BOC-N $_{\epsilon}$ -Tos- β -homoarginine was hydrolysed in HF and used as a standard for amino acid analysis. The retention time of β -homoarginine was identical to that of arginine, but the intensity of the peak was considerably lower, as expected.

N $^{\alpha}$ -BOC-N $_{\epsilon}$ -Tos- β -Homoargininylglycine Benzylester

We next combined 4.06 g (9.2 mmole) of the above carboxylic acid with 2.04 ml of N-methylmorpholine in 25 ml of anhydrous THF. The mixture was stirred under argon at -5° C. A chilled solution of isobutylchloroformate (2.4 ml in 25 ml THF) was then added dropwise to the solution over 10 minutes. Following this addition, the reaction mixture was stirred for 12 minutes at -5° C. For Hirulog-18a we then added a solution of glycine benzyl ester (4.9 g in 40 ml THF; 27.6 mmole), and allowed the reaction mixture to come to room temperature. The solvent was then removed in vacuo and the resulting residue dissolved in 100 ml ethylacetate. The solution was extracted successively with 100 ml each of saturated NaHCO $_3$ and saturated NaCl, dried over MgSO $_4$, and concentrated in vacuo. The resulting crude dipeptide ester was purified on a 4 \times 20 cm silica gel column with a methanol step gradi-

ent in chloroform containing 10 drops NH OH per 100 ml (2 l of 1% methanol in chloroform, followed by 3 l of 2% methanol in chloroform). Fractions (25 ml) were collected, assayed by TLC and those containing product were pooled and the solvent removed in vacuo. The resulting product, N^α-BOC-N^ε-Tos-β-homoargininylglycine benzylester, was isolated a white foam (3.9 g).

For Hirulog-18b and -18c, the above reaction was identical except for the following modifications: For Hirulog-18b, the glycine benzyl ester was replaced by proline benzyl ester and the reaction was run on a 1.8 mmole scale. For Hirulog-18c, the glycine benzyl ester was replaced with valine benzyl ester and the reaction was run on a 3.0 mmole scale.

N^α-BOC-N^ε-Tos-β-Homoargininylglycine

The above benzyl ester was dissolved in 50 ml methanol and hydrogenated at atmospheric pressure over 1.0 g of 10% palladium/carbon for 17 h. The resulting solution was filtered through Celite and the solvent removed in vacuo. The reaction yielded 2.9 g of crude N^α-BOC-N^ε-Tos-β-homoargininylglycine, which was purified on a Vydac C₁₈ HPLC column as described above.

The above N^α-BOC-N^ε-Tos-β-homoargininylglycine (1.02 g) was dissolved in 1 ml anhydrous DMF and cooled in an ice bath. We then added to this solution successively, 5.5 ml of 0.5M hydroxybenztriazole in DMF (Applied Biosystems Inc, Foster City, Calif.) and 5.5 ml of 0.5M dicyclohexylcarbodiimide in CH₂Cl₂ (Applied Biosystems). After 1 hour, the cold suspension of symmetrical anhydride of the dipeptide unit was then rapidly filtered through a plug of glass wool to remove the dicyclohexyl urea.

Meanwhile, a suspension of N-BOC-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-O-PAM (0.2 mmol in CH₂Cl₂) was activated by standard peptide synthesis methods. A Kaiser test on the resulting product indicated a free terminal amino group.

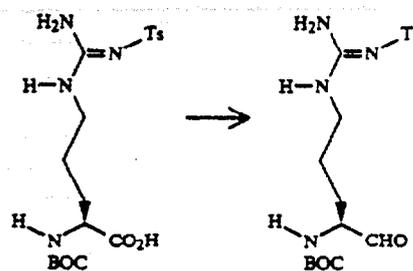
The activated β-homoargininylglycine dipeptide was then coupled to the resin-bound hexadecapeptide. The resulting octadecapeptide was then coupled, successively, with N-BOC-Pro and N-BOC-(D-Phe) using standard coupling procedure. The resulting peptide, Hirulog-18a, was purified and characterized as described in Example 4.

A similar protocol was carried out for the synthesis of Hirulog-18b and Hirulog-18c.

EXAMPLE 22

Synthesis Of Hirulog-19

Hirulog-19 has the formula: H-(D-Phe)-Pro-Arg-[psiCH₂NH]-(Gly)₅-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. Residues 4-20 of this peptide were assembled by solid-phase peptide synthetic procedures as described in Example 4. The next residue added, N^α-BOC-N^ε-tosyl-argininal, was prepared as depicted and described below.



N^α-BOC-N^ε-Tos-Argininal

N^α-BOC-N^ε-Tos-arginine (Bachem Inc.; 10 g) was added to 80 ml of anhydrous THF and the suspension cooled to 0°-5° C. We then added 1,1'-carbonyldiimidazole (Aldrich; 3.61 g) all at once and continued stirring for 20 minutes. The resulting clear solution was partially immersed in a dry ice/acetone bath to maintain a temperature of -20° to -30° C. during the dropwise addition of a suspension of lithium aluminum hydride (Aldrich; 1.8 g in 80 ml THF) over 45 minutes with constant stirring. The reaction was stirred an additional 30 minutes at -20° C. and was then quenched by the dropwise addition of 63 ml of 2N HCl at -10° C. We filtered the resulting solution through a medium sinter glass funnel and concentrated the resulting filtrate in vacuo.

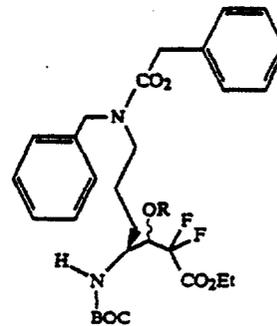
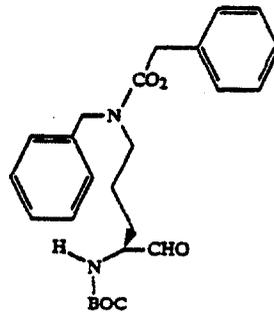
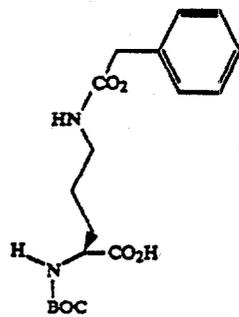
The resulting crude aldehyde, recovered as a white foam (11.5 g), was suspended in 100 ml of chloroform, washed with water (2×50 ml) and the organic layer then dried over sodium sulfate and concentrated in vacuo. The crude aldehyde (7.7 g) was dissolved in 100 ml chloroform and purified by flash chromatography over a 5×20 cm flash column containing 350 ml silica gel (Merck Grade 60, 230-400 mesh, 60 Å). Elution was achieved using a step gradient of 0.5% methanol in 500 ml chloroform, 1% methanol in 1 l chloroform, and 1.5% methanol in 1 l chloroform. This procedure yielded 8.9 g of N^α-BOC-N^ε-Tos-argininal.

The N^α-BOC-N^ε-Tos-argininal (258 mg) was then added to the resin-bound (Gly)₅-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-O-PAM under solid-phase reductive alkylation conditions (40 mg sodium cyanoborohydride for 24 hours) using the method of D. H. Coy et al., "Solid-Phase Synthesis of Peptides" In Peptides, Vol. 8, pp. 119-121 (1978). Following reaction of the resin-linked peptide with the protected argininal, the peptide synthesis was completed with a cycle of BOC-proline incorporation and a cycle of BOC-(D-phenylalanine) incorporation. After completion of the synthesis, Hirulog-19 was deprotected and uncoupled from the resin as described in Example 4.

Hirulog-19 was purified by reverse phase HPLC employing an Applied Biosystems 151A liquid chromatographic system and an Aquapore C₈ column (10×22 cm). The column was equilibrated in 1 part 70% acetonitrile/30% water containing 0.85% TFA (Buffer B) and 4 parts water containing 1% TFA (Buffer A). The column was developed with a linear gradient of increasing Buffer B concentration (20-50%) over 120 minutes at a flow rate of 4.0 ml/minute. The effluent stream was monitored for absorbance at 214 nm and fractions were collected manually. Further purification was carried out under isocratic conditions using 20 Buffer B/80% Buffer A.

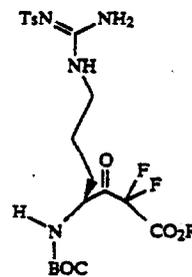
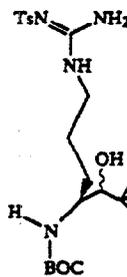
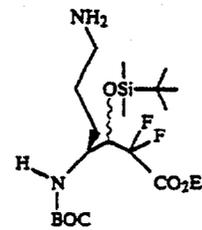
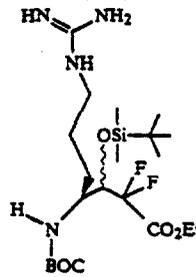
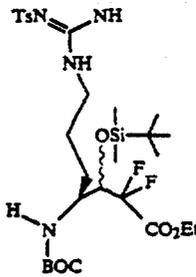
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EXAMPLE 23



R = H

R = Si



R = Et

R = H

Synthesis Of Hirulog-21

Hirulog-21 has the formula: H-(D-Phe)-Pro-Arg-Pro-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-(Gly)₂-Lys-OH. Hirulog-21 was synthesized using methods described in Example 4, using the appropriate BOC-amino acids. Purification and characterization of Hirulog-21 were achieved by the methods described in Example 4.

EXAMPLE 24

Synthesis Of Hirulog-25

Hirulog-25 has the formula H-(D-Phe)-Pro-(4-Arginyl-2,2-difluoro)malonylglycyl-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. The hexadecapeptide, (Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu, was synthesized as previously described and left bound to the resin. The next residue, (3-Arginyl-2,2-difluoro)malonylglycine is

synthesized in the reaction scheme depicted and detailed below.

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1-[(2'-carboethoxy 1', 1'-difluoro)ethyl]N^α-BOC-N^ω-benzyl-N^ω-CbzOrnithinol

A solution of 3.1 g (7.1 mmoles) N^α-BOC-N^ω-Benzyl-N^ω-CbzOrnithinol [F. Salituro et al., "Inhibition of Aspartic Proteinases By Peptides Containing Lysine and Ornithine Side Chain Analogues of Statine", *J. Med. Chem.*, 30, pp. 286-95 (1987)], and 1.56 ml (9.23 mmoles) ethylbromodifluoroacetate in anhydrous 15 ml THF was added over 90 minutes to a refluxing suspension of 786 mg Zn powder (Fluka) in 15 ml THF under argon. After 4 hours of reflux and 2 hours at room temperature, the mixture was cooled and partitioned between 200 ml each of ethyl acetate and saturated NaCl/KHSO₄. The organic phase was isolated, dried over MgSO₄ and concentrated in vacuo. The resulting oily residue was purified on silica gel, using CHCl₃:methanol (90:10) plus 100 drops/1 NH₄OH as eluant.

1-[(2'-Carboethoxy-1',1'-difluoro)ethyl]N^α-BOC-Ornithinol tertButyldimethylsilyl Ether

The resulting compound, 1-[(2'-carboethoxy-1'-1'-difluoro)ethyl]N^α-BOC-N^ωm-benzyl-N^ωm-CbzOrnithinol, is then reacted with 5 equivalents of tert-butyl-dimethylsilyl chloride and 10 equivalents of imidazole in anhydrous DMF at 35° C., following the procedure of E. J. Corey et al., "Protection of Hydroxyl Groups as tertButyldimethylsilyl Derivatives", *J. Amer. Chem. Soc.* 94, pp. 6190-91, (1972). The orthogonally protected amine is then dissolved in methanol and hydrogenated over Pd(OH), at 30 psi for 18 hours. The catalyst is then removed by filtration and the filtrate concentrated in vacuo to produce 1-[(2'-carboethoxy-1'-1'-difluoro)ethyl]N^α-BOC-Ornithinoltert-butyl-dimethylsilyl ether.

1-(2'-Carboethoxy-1',1'-difluoro)ethyl]N^α-BOC-N^ω-Tos-Aroininol-tertButyldimethylsilyl Ether

The above-prepared compound is then reacted with 6.8 equivalents each of 1-guanyl-3,5-dimethylpyrazole and triethylamine in water at 105° C. for 24 hours. The mixture is then lyophilized and the residue subjected to preparative HPLC as described in Example 4. Fractions containing the desired guanidinium compound (assayed by TLC) are pooled and dried in vacuo. The residue is dissolved in H₂O:acetone (1:4), cooled in an ice bath and adjusted to pH 12 with 50% w/v NaOH. To this solution we add a solution of 3 equivalents of oarotoluene sulfonylchloride in acetone over 60 minutes, while maintaining the pH at 11-12 with NaOH. The solution is allowed to warm to room temperature and is stirred overnight. The acetone is then removed in vacuo and the remaining aqueous solution is washed with ether. The ether layer is removed and back extracted with saturated NaHCO₃. The aqueous phases are combined and acidified to pH 3 with 2N HCl. The resulting acid solution is then extracted two times with ethyl acetate, dried and concentrated in vacuo to yield the desired product.

1-[(2'-Carboxy-1',1'-difluoro)ethyl]N^α-BOC-N^ω-Tos-Argininol

The resulting compound, 1-[(2'-carboethoxy-1'-1'-difluoro)ethyl]N^α-BOC-N^ω-Tos-Argininoltert-butyl-dimethylsilyl ether, is desilylated by treatment with 3 equivalents of tetra-n-butylammonium fluoride in THF

at room temperature, as described in E. J. Corey et al., supra. The compound produced by this process is then saponified by treatment with 2.5 equivalents of LiOH in methanol/water at room temperature overnight under argon. The reaction mixture is then washed with ethyl acetate and acidified with citric acid to pH 4. We extract the resulting acid into ethyl acetate, dry the organic phase and concentrate it in vacuo. The crude acid is then purified on a Vydac C₁₈ reverse-phase HPLC column under the conditions described in Example 4.

1-[(2'-Carboxy-1',1'-difluoro)ethyl]N^α-BOC-N^ω-Tos-Argininone

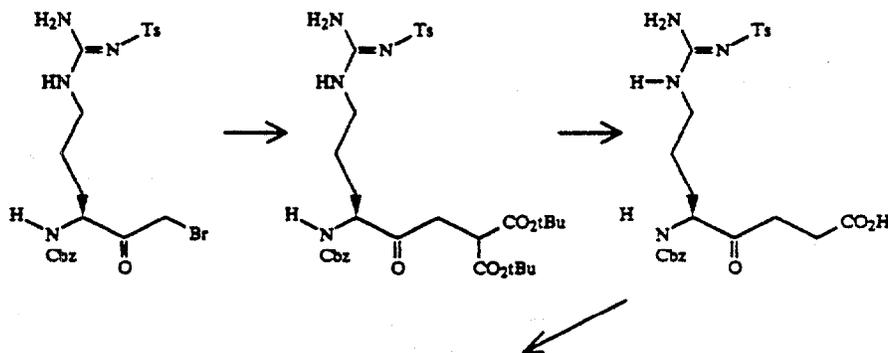
The alcohol function of the above compound is converted to the ketone by the addition of one equivalent of pyridinium dichromate in CH₂Cl₂ containing 0.5% glacial acetic acid in the presence of molecular sieves [N. Peet et al., "Synthesis of Peptidyl and Fluoromethyl Ketones and Peptidyl α-Keto Esters as Inhibitors of Porcine Pancreatic Elastase, Human Neutrophil Elastase, and Rat and Human Neutrophil Cathepsin G", *J. Med. Chem.*, 33, pp. 394-407 (1990)]. After stirring under argon for 15 hours, the reaction mixture is filtered and the solvent removed in vacuo. The resulting 1-[(2'-carboxy-1',1'-difluoro)ethyl]N^α-BOC-N^ω-Tos-Argininone is recovered as an oily residue and then purified on HPLC according to the conditions specified in Example 4.

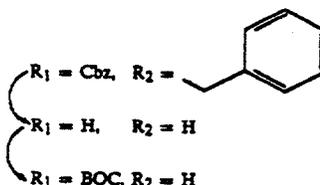
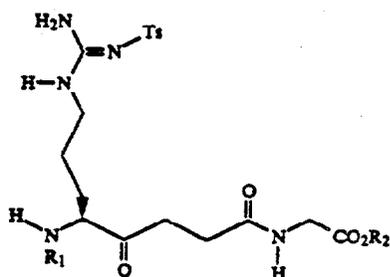
The free carboxylic acid is converted to the symmetrical anhydride and reacted with resin-bound hexadecapeptide as described in Example 21. The two N-terminal residues of Hirulog-25, BOC-Pro and BOC-(D-Phe), are added under standard peptide synthesis conditions and the resulting peptide is then cleaved with HF.

EXAMPLE 25

Synthesis Of Hirulog-26

Hirulog-26 has the formula: H-(D-Phe)-Pro-Argoxopropionylglycyl-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. The hexadecapeptide, (Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu, was synthesized as previously described and left bound to the resin. The next residue, N^α-BOC-argoxopropionylglycine, is synthesized by the reaction scheme depicted and described below.





-continued

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3-(CbzAmino)-2-oxo-3-{3-[(N^ε-Tos)guanidiny]propyl}-di-tertButylMalonate

We prepared a batch of N^α-Cbz-N^ε-Tos-ArginineDiazomethyl ketone in the same manner as the preparation of N^α-BOC-N^ε-Tos-ArginineDiazomethyl ketone described in Example 21, except for the substitution of N^α-Cbz-N^ε-Tos-Arginine for N^α-BOC-N^ε-Tos-ArginineDiazomethyl ketone in 200 ml of CH₂Cl₂ in a flask and cooled the solution to -70° C. in a dry ice/acetone bath with stirring. Anhydrous HBr gas was then bubbled through the solution at a moderate flow rate for 15 minutes. The solution was stirred for an additional 15 minutes at -70° C. and then concentrated in vacuo. The resulting product, N^α-Cbz-N^ε-Tos-Arg-COCH₂Br, was recovered as 5.0 g of yellow crystals.

Meanwhile, a suspension of sodium hydride (36 mg; 80% dispersion in oil) in 1 ml DMF and 1.2 ml hexamethylphosphoramide ("HMPA") was added to a solution of 259 mg di-tertbutoxymalonate in 4 ml DMF. The mixture was stirred at room temperature for 40 minutes and was then added dropwise, over 20 minutes, to a solution of 1 mmole N^α-Cbz-N^ε-Tos-Arg-COCH₂Br, in 1 ml DMF/0.13 ml HMPA. The reaction was allowed to proceed for 3 hours, after which time the solution was poured into 50 ml water and extracted with 2 × 50 ml ethyl acetate. The organic phase was isolated, dried and concentrated in vacuo to an oily residue. The residue was subsequently purified on a 3 × 10 cm silica gel column which was eluted successively with 400 ml of 5% acetone in chloroform, 400 ml of 10% acetone in chloroform and 200 ml of 20% acetone in chloroform. Fractions (25 ml) were collected and assayed by TLC. Fractions containing the desired product were pooled and concentrated to produce 3-(CbzAmino)-2-oxo-3-{3-[(N^ε-Tos)guanidiny]propyl}-di-tertButyl malonate.

5-(N^α-CbzAmino)-4-oxo-5-{3-(N^ε-Tos)guanidiny]propyl}pentanoylglycine Benzyl Ester

The above di-tert butyl ester is stirred in 1.2 equivalents of 1N HCl for 2 hours at room temperature. It is then decarboxylated in excess pyridine at 100° C. for 15 minutes. The solvent is then removed in vacuo, and the residue purified by silica gel chromatography, as described above. The resulting carboxylic acid is acylated

with glycine benzyl ester according to the method described in Example 21.

5-(Amino)-4-oxo-5-{3-(N^ε-Tos)guanidiny]propyl}pentanoylglycine

The resulting ester is dissolved in 500 ml methanol and hydrogenated overnight at 1 atmosphere of hydrogen gas over 600 mg of 10% palladium-carbon catalyst. The reaction mixture is then filtered through Celite and concentrated in vacuo to a solid residue (155 mg). The resulting amino acid is then purified by HPLC₈ using the conditions described in Example 4.

5-(N^α-BOCAmino)-4-oxo-5-{3-[(N^ε-Tos)guanidiny]propyl}pentanoylglycine

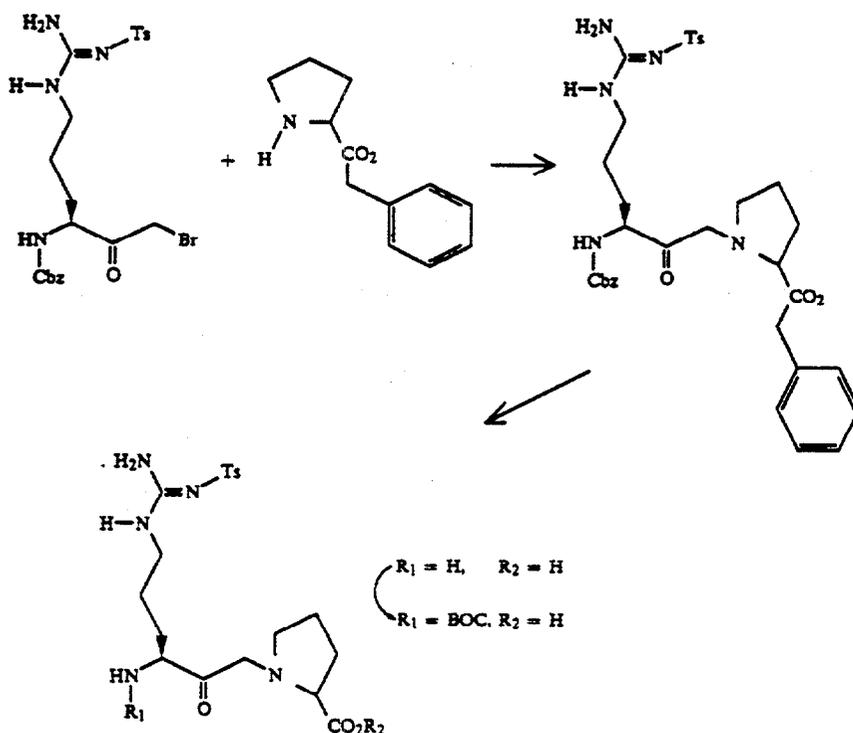
The above amino acid is converted to its corresponding BOC derivative by dissolving in dioxane/water (2:1, v/v) and cooling to 0° C. with stirring. The pH is adjusted to 10 with 0.1N NaOH and then 1.1 equivalents of di-tert-butyl dicarbonate (in dioxane) are added. The reaction is stirred at 0° C. to 20° C. for 4 hours and then is evaporated in vacuo. The residue is then partitioned between ethyl acetate/1% citric acid (2:1). The organic phase is isolated, extracted once with 1% citric acid, and then 3 times with saturated NaCl. The organic phase is dried over MgSO₄, filtered and concentrated in vacuo to obtain the BOC-protected product.

The resulting protected pseudopeptide free carboxylate is then coupled to the resin-bound hexadecapeptide using standard peptide synthesis techniques. This is followed by the sequential addition of BOC-D-Phe and BOC-Pro to the resin-bound peptide. The completed Hirulog-26 is then cleaved from the resin, deprotected and purified as described in Example 4.

EXAMPLE 26

Synthesis of Hirulog-27

Hirulog-27 has the formula H-(D-Phe)-Pro-Arg-(CO-CH₂)-Pro-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. The (Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu hexadecapeptide was synthesized as previously described and left bound to the resin. The remaining portion of the molecule was synthesized by the reaction scheme depicted and described below.



N^α-Cbz-N^ε-Tos-Arginine(COCH₂)proline Benzyl Ester

We dissolved 720 mg of proline benzyl ester (HCl salt) in 25 ml THF. This solution was then cooled to -78° C. in an acetone/dry ice bath with stirring under argon. We then added lithium diisopropylamide (8.0 ml of a 0.75 M hexane suspension) and stirred for an additional 5 minutes. To this we added 1.08 g N^α-Cbz-N^ε-Tos-ArginineBromomethyl Ketone in 10 ml THF, prepared as described in Example 25, dropwise over 20 minutes. The reaction was stirred for an additional 5 minutes and the solution was then allowed to warm to room temperature with stirring. We quenched the reaction by adding 10 ml of saturated NaCl, allowed the phases to separate and isolated the organic phase. This phase was then dried over MgSO₄, filtered and evaporated in vacuo.

N^α-BOC-N^ε-Tos-Arginine(COCH₂)proline

The above benzyl ester (1.3 g) was hydrogenated using the palladium-carbon procedure described in Example 25. The resulting pseudodipeptide was BOC-protected by the procedure described in Example 25 to produce the desired product.

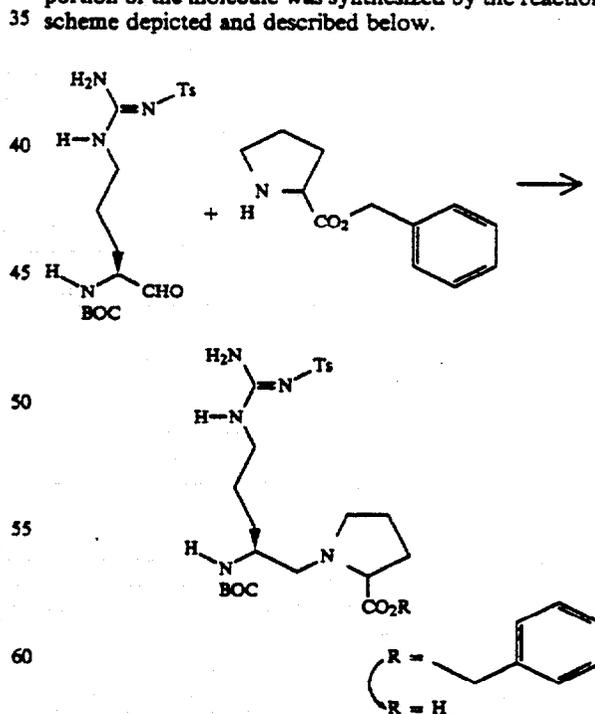
The purified, protected pseudodipeptide was then coupled with the resin-linked hexadecapeptide by standard peptide synthesis techniques. Hirulog-27 was deprotected, cleaved from the resin and purified by the techniques described in Example 4.

EXAMPLE 27

Synthesis Of Hirulog-28

Hirulog-28 has the formula: H-(D-Phe)-Pro-Arg(CH₂N)-Pro-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. The (Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-O-PAM hexadecapeptide was synthesized as previously

described and left bound to the resin. The remaining portion of the molecule was synthesized by the reaction scheme depicted and described below.



N^α-BOC-N^ε-Tos-Arginine[psiCH₂N]Proline Benzyl Ester

One gram of crushed 3 Å molecular sieves (Aldrich) was added to a stirred solution of 5.25 g proline benzyl

ester free base (Schweizerhall, Inc.) in 10 ml anhydrous THF and 2 ml anhydrous ethanol under argon at room temperature. We added 1.45 ml of 5N methanolic HCl and 1.5 g of N^α-BOC-N^ε-Tos-Argininal (prepared as described in Example 22) to this mixture and stirred for 1 hour. An 85 mg portion of sodium cyanoborohydride was added to the mixture and then, an hour later, a second 85 mg portion of sodium cyanoborohydride was added. The reaction was then stirred for 20 hours and filtered. We added 1 ml water and 0.9 ml 1 N HCl to the filtrate with stirring and then concentrated the solution in vacuo to yield 6.2 g of N^α-BOC-N^ε-Arg[psiCH₂H]-Pro-benzyl ester, as a clear oil.

The oil is further purified by flash chromatography over a 5 cm flash column containing 350 ml silica gel (Merck Grade 60, 230-400 mesh, 60 Å). The product was obtained by successive elution with 0.25%, 0.75% and 1.5% methanol in chloroform.

N^α-BOC-N^ε-Tos-Arginine[psiCH₂N]Proline

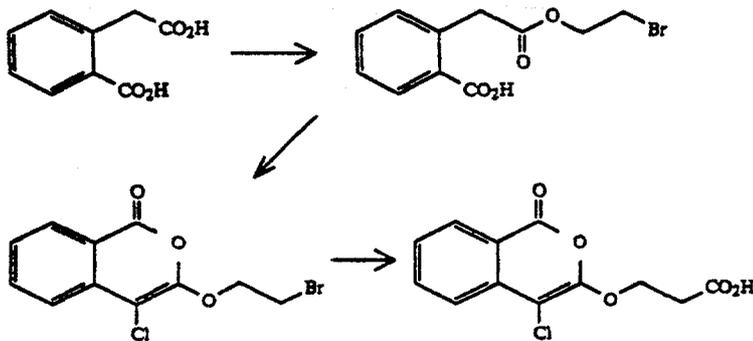
The resulting benzyl ester is hydrogenated over palladium-carbon and purified, as described in Example 25. This process yielded 160 mg of N^α-BOC-N^ε-Arg[psiCH₂H]-Proline free acid, which was further purified using the HPLC chromatography system described in Example 4, except elution was achieved with an isocratic 26% Buffer B/74% Buffer A system, previously described in Example 22. The final yield of dipeptide was 86 mg.

The dipeptide is then coupled to the resin-bound hexadecapeptide, followed by a cycle of BOC-Pro incorporation and a cycle of BOC-(D-Phe) incorporation. Deprotection, cleavage and purification of the fully synthesized Hirulog-28 is achieved by the method described in Example 4.

EXAMPLE 28

Synthesis Of Hirulog-29

Hirulog-29 has the formula: 4-chloro-isocoumarino-3-carboxyethoxy-(Gly)₅-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. The (Gly)₅-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu heptadecapeptide was synthesized as previously described and left bound to the resin. The 4-chloroisocoumarino-3-carboxyalkoxy moiety was synthesized by the reaction scheme and methods described below.



Ethyl 2-bromo-Homophthalate

We mixed homophthalic acid (10.0 g), 2-bromoethanol (21.0 g) and benzene (200 ml). We then added 12-15 drops of sulfuric acid and heated to reflux for 2.5 hours. The solution was then filtered and concentrated in

vacuo. The residue was washed with 250 ml ether/hexane (1:1) and was filtered onto a scintered glass funnel. The resulting light brown solid was vacuum dried to obtain approximately 15.0 g of product.

4-chloro-3-[2-bromoethoxy]-isocoumarin

We mixed the ethyl 2-bromo homophthalate prepared as described above (4 g) together with phosphorous pentachloride (8.2 g) and benzene (100 ml). The mixture was refluxed for 4.5 hours, filtered hot and evaporated in vacuo. The reddish-brown oily residue was chromatographed immediately on a 24 mm x 175 mm silica gel column using dichloromethane as eluant. Fractions of 20 ml were collected and assayed by TLC. The 4-chloro-3-[2-bromoethoxy]-isocoumarin eluted in fractions 2-6. The fractions were pooled, evaporated in vacuo and the resulting residue was recovered as a clear, light yellow oil (2.2 g).

4-chloro-3-[3-oxopropanoic acid]-isocoumarin

The 4-chloro-3-[2-bromoethyl]-isocoumarin (1.4 g) prepared above was dissolved in anhydrous THF and added directly to a refluxing solution of magnesium turnings (170 mg), and a few crystals of iodine in 15 ml anhydrous THF, which was stirring under argon. The mixture was refluxed for 1.5 hours. It was then poured over excess dry ice in a 400 ml beaker. We let the mixture stand at 20° until all the excess CO₂ had sublimed and then added approximately 100 ml each of diethyl ether and THF to the mixture which produced a yellow solution containing a large amount of white, coarse precipitate.

We bubbled anhydrous HCl through this mixture at 20°, which dissolved most of the precipitate. The solution was then filtered and evaporated in vacuo to obtain the crude product. This was then recrystallized overnight from DCM.

The resulting 4-chloro-3-[3-oxopropanoic acid]-isocoumarin is coupled to a glycine benzyl ester and the resulting product catalytically hydrogenated over palladium-carbon, as described in Example 25. This pseudodipeptide is then coupled to the resin-bound hexadecapeptide, (Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu, by standard peptide synthesis techniques.

EXAMPLE 29

Synthesis Of Hirulog-30

Hirulog-30 has the formula: 4-chloro-3-[2-aminoethanol]-isocoumarin-(Gly)₅-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu. The hexadecapeptide,

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(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu is synthesized as previously described and left bound to the resin.

The 4-chloro-3-[2-aminoethanol]-isocoumarin moiety is prepared by a procedure analogous to that described in Example 28 for synthesizing 4-chloro-3-[2-bromoethanol]-isocoumarin, except that 2-aminoethanol is used instead of 2-bromoethanol in the initial step of esterifying homophthalic acid.

The urea linkage is formed by reacting the amino group of 4-chloro-3-[2-aminoethanol]-isocoumarin with the activating agent, carbonyldiimidazole ("CDI"). The resulting intermediate imidazolidine is not isolated, but is reacted with the resin-linked hexadecapeptide to produce Hirulog-30. Hirulog-30 is then deprotected,

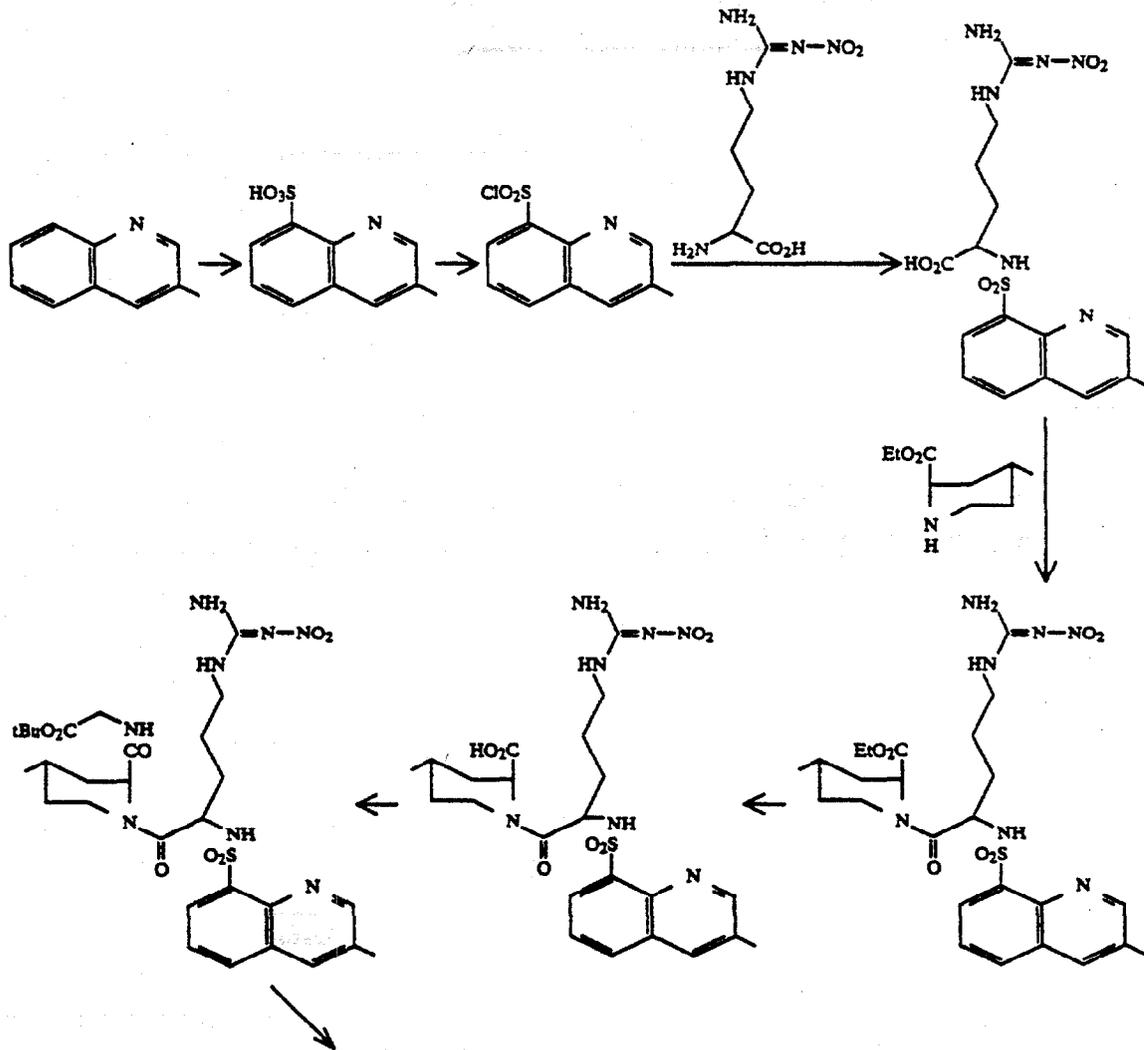
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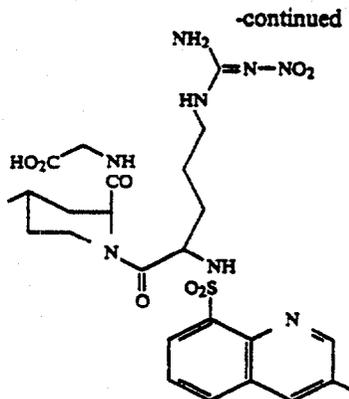
cleaved from the resin and purified by the techniques described in Example 4.

EXAMPLE 30

Synthesis Of Hirulog-31

Hirulog-31 has the formula argipidyl-(Gly)₅-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. We synthesized the hexadecapeptide (Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu by the standard peptide synthesis techniques described previously and leave the peptide bound to the resin. The argipidylglycine portion of this Hirulog is synthesized by the reaction scheme depicted and described below.





A Dehydro-N-NitroArgipidine is synthesized essentially by the method for synthesizing argipidine, which is described in U.S. Pat. No. 4,258,192, herein incorporated by reference. The only differences are that the guanidinium group is protected by a nitro function and the heterocyclic ring of the quinoline remains unsaturated. The intermediate is used to acylate t-butyl glycine by the method described in Example 21. The t-butyl ester is removed by standard acid hydrolysis techniques. The resulting free acid is reacted with the hexadecapeptide using standard coupling techniques. The resultant peptide is deprotected, cleaved from the resin and purified by the techniques described in Example 4.

The peptide is then subjected to the hydrogenation procedure described in the 4,258,192 patent and purified by the HPLC technique described in Example 4.

EXAMPLE 31

Synthesis Of Hirulog-32

Hirulog-32 has the formula: H-(D-Phe)-Pro-Arg-(Gly)₅-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. Hirulog-32 was synthesized, purified and characterized using the methods described in Example 4, except that BOC-glycine was used instead of BOC-proline in the cycle following the two cycles of BOC-glycylglycine addition.

EXAMPLE 32

Synthesis Of Hirulog-33

Hirulog-33 has the formula: N-acetyl-Gly-Asp-Phe-Leu-Ala-Glu-(Gly)₃-Val-Arg-Pro-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH. Hirulog-33 was synthesized, purified and characterized by the standard peptide synthesis techniques employed in Example 4, with appropriate BOC-amino acid substitutions. The CSDM portion of Hirulog-33 has an amino acid sequence that is identical to a segment of the fibrinopeptide A sequence of the A α chain in human fibrinogen.

EXAMPLE 33

Cleavage Of Various Hirulogs By Thrombin

Inhibition of thrombin by Hirulog-8 was found to be transient due to the slow cleavage of the Arg-Pro bond by thrombin. Following this cleavage, thrombin was observed to recover full hydrolytic activity toward a chromogenic substrate. Therefore, Hirulog-8 was characterized as a "slow-substrate" inhibitor of thrombin.

The cleavage of Hirulog-8, as well as other Hirulogs of this invention, by human α -thrombin was demon-

strated in *in vitro* assays. Reaction mixtures containing human α -thrombin (1.6 nM) and varying concentrations of either Hirulog-8, Hirulog-10, Hirulog-18a, Hirulog-18b, Hirulog-18c, Hirulog-19, Hirulog-32 or Hirulog-33 (80 to 160 nM) were prepared in 20 mM Tris-HCl, pH 7.4 containing 0.1M NaCl. Aliquots (0.975 ml) of the reaction mixtures were removed at various times and mixed in a cuvette with 0.025 ml Spectrozyme TH (11 μ M final concentration), a chromogenic substrate. The initial rate of reaction was determined and, based on control mixtures containing thrombin in the absence of Hirulog, the % inhibition was calculated.

An alternate method employed reverse-phase HPLC separation of aliquots from a Hirulog/thrombin reaction mixture. In this assay we added human α -thrombin (0.25 μ M final concentration) to a reaction vessel containing one of the above Hirulogs (12.5 μ M final concentration). Aliquots (5 μ l) were removed both prior to and at various times following the addition of thrombin. The aliquots were either flash frozen or injected directly onto the HPL column. The HPLC system employed an Applied Biosystems Liquid Chromatography System equipped with an Aquapore C₈ column (0.46 \times 10 cm). The column was equilibrated in 70% solvent A (0.1% TFA in water) and 30% solvent B (0.085% TFA/70% acetonitrile) and developed with a linear gradient of from 30 to 50% solvent B over 30 minutes at a flow rate of 1 ml/minute. The effluent stream was monitored at 214 nm. Peptide concentrations were determined by measurement of peak heights.

Both of the above-described assays allow determination of the rate of Hirulog hydrolysis by thrombin (expressed in M/min) and turnover rate (k_{cat} , expressed in min⁻¹). Both methods produced comparable k_{cat} values, which are shown in the table below.

INHIBITOR	P ₁ -P ₁ ' SEQUENCE	k_{cat} (min ⁻¹)
Hirulog-8	Arg-Pro	0.31-0.5
Hirulog-10	Arg-Sar	10
Hirulog-18a	β -HomoArg-Gly	<0.01
Hirulog-18b	β -HomoArg-Pro	<0.01
Hirulog-18c	β -HomoArg-Val	<0.01
Hirulog-19	Arg[psiCH ₂ NH]-Gly	<0.01
Hirulog-32	Arg-Gly	535
Hirulog-33	Arg-Pro	0.056

As shown above, Hirulog-8, -10, -32 and -33 were cleaved by thrombin with k_{cat} values ranging from 0.056 min⁻¹ (slow cleavage) to 535 min⁻¹ (fast cleavage). In

contrast, Hirulog-18a, -18b, -18c, and -19 appear to be resistant to thrombin cleavage.

FIG. 5, panels A and B, show a more detailed analysis of the cleavage of Hirulog-8 by thrombin. As depicted in FIG. 5, panel A, concentrations of Hirulog-8 in slight excess over thrombin exhibited a transient inhibitory activity (greater than, or equal to, 10 minutes, depending on the Hirulog concentration). Progressively higher concentrations of Hirulog-8 demonstrated prolonged inhibitory effects. A linear relationship between duration of inhibition and Hirulog-8 concentration is shown in FIG. 5, panel B. From these data, we calculated a turnover time, or k_{cat} of 0.37 min^{-1} .

By purification and sequence analysis of the Hirulog-8-derived digestion products produced in the reactions above, we determined that Hirulog-8 was slowly cleaved by thrombin at the Arg-Pro bond. This is a highly unusual cleavage site for serine proteases and we believe it to be susceptible to cleavage in Hirulog-8 due to the high affinity of the peptide for thrombin.

EXAMPLE 34

The Effect Of Linker Length On The Activity Of Hirulog

Hirulog-8, Hirulog-13, Hirulog-15, and Hirulog-16 differ from one another only by the length of the polylucine portion of their respective linker segments. In order to determine what effect linker length has on activity, we compared the inhibition of human α -thrombin by each of these Hirulogs. The following table lists the linker lengths of each of these Hirulogs:

Peptide	Linker Length (Å)
Hirulog-8	24
Hirulog-13	18
Hirulog-15	30
Hirulog-16	36

The antithrombin activities of these Hirulogs was measured toward thrombin-catalyzed hydrolysis of Spectrozyme TH essentially as described in Example 9. FIG. 6 depicts the relationship of linker length to K_1 for Hirulog inhibition of this thrombin-catalyzed reaction. This figure shows that Hirulogs-8, -15 and -16 have comparable inhibitory activities, while Hirulog-13, with an 18 Å linker length, has an activity reduced by more than 10-fold. This confirms that linker lengths of $> 18 \text{ Å}$ and $< 42 \text{ Å}$ do not affect Hirulog activity. While not wishing to be bound by theory, applicants believe this is due to the fact that the Hirulog linker is equally disordered when free in solution as when bound to thrombin. Applicants also believe that there is little cooperativity in the binding of the CSDM and ABEAM portions of the thrombin inhibitors of this invention to thrombin.

EXAMPLE 35

Inhibition Of Thrombin-Catalyzed Hydrolysis By Various Hirulogs

We compared the inhibitory activity of various thrombin inhibitors of the present invention on thrombin-catalyzed hydrolysis of a tripeptidyl-p-nitroanilide substrate. The antithrombin activities of Hirulog-10, Hirulog-18a, Hirulog-18b, Hirulog-18c, Hirulog-19, Hirulog-32 and Hirulog-33 were assayed by the method described in Example 9, using Spectrozyme TH as a substrate. The table below lists the calculated K_1 values

as well as the P_1-P_1' sequence, of each of these thrombin inhibitors.

INHIBITOR	P_1-P_1' SEQUENCE	K_1 (nM)
Hirulog-8	Arg-Pro	1.9 ± 1.4
Hirulog-10	Arg-Sar	$> 2,000$
Hirulog-18a	β -HomoArg-Gly	7.4
Hirulog-18b	β -HomoArg-Pro	4.6
Hirulog-18c	β -HomoArg-Val	205.0
Hirulog-19	Arg[psiCH ₂ NH]-Gly	20.0
Hirulog-32	Arg-Gly	$> 2,000$
Hirulog-33	Arg-Pro	3.6

As indicated above, Hirulog-10 and Hirulog-32 were poor inhibitors of thrombin-catalyzed hydrolysis of Spectrozyme TH. This was consistent with the fact that each of these inhibitors was rapidly cleaved by thrombin at the P_1-P_1' bond. In Hirulog-19, wherein this bond was reduced to the psiCH₂-NH linkage and rendered non-cleavable by thrombin, effective inhibition of thrombin hydrolysis was observed.

The studies with β -homoarginine-containing inhibitors (Hirulogs-18a, -18b and -18c) demonstrated that this amino acid derivative may replace arginine in the inhibitors of this invention without affecting activity. Moreover, this shows that displacement of the amide bond by one methylene does not markedly reduce thrombin inhibitory activity. The 30- to 50-fold increase in K_1 for Hirulog-18c, as compared to Hirulog-18a and -18b, respectively, suggests that the structure of the P_1 amino acid is important in inhibitory activity. Without wishing to be bound by theory, applicants believe that the presence of phi-psi angles in the P_1 amino acid (Gly in Hirulog-18a; Pro in Hirulog-18b) as well as conformational constraints, (such as is caused by the proline in Hirulog-18b) contribute to the potency of the inhibitors of this invention. An alternate possibility is that the β -branched side chain of the P_1 amino acid Val in Hirulog-18c may impair binding of the CSDM portion of that molecule to the thrombin reactive center due to steric considerations.

EXAMPLE 36

Binding Of Hirulog-8 To The Active Site Of Thrombin

Diisopropylfluorophosphate (DFP) is a well-known inhibitor of serine proteases, including thrombin, which acts by covalently modifying the hydroxyl group of Ser-195. We added a 270-fold excess of ¹⁴C-DFP to thrombin, in 0.1M sodium borate, pH 8.0. Following a 10 minute reaction, formation of a thrombin complex was demonstrated by SDS-PAGE and fluorographic analyses (FIG. 7, lane 1). When the reaction was performed in the presence of Sulfo-Tyr₆₃-N-acetylhirudin₅₃₋₆₄ (at 300 and 3000-fold molar excess over thrombin), the modification of thrombin by [¹⁴C]-DFP was not altered significantly (FIG. 7, lanes 4 and 5). However, when we performed the reaction in the presence of Hirulog-8 (at 3- or 30-fold molar excess over thrombin) the incorporation of [¹⁴C]-DFP into the thrombin catalytic site was completely blocked (FIG. 7, lanes 2 and 3). These data demonstrate that the CSDM of the thrombin inhibitors of this invention are capable of binding to the catalytic site of thrombin and inhibiting catalytic activity.

EXAMPLE 37

Comparison Of Antithrombin Activity Of Hirulog-8
And A Synthetic Catalytic Site Directed Pentapeptide
(D-Phe-Pro-Arg-Pro-Gly)

As shown in FIG. 1, Hirulog-8, unlike its constituent anion-binding exosite associating moiety, Sulfo-Tyr₆₃-N-acetyl-hirudin₅₃₋₆₄, was able to inhibit thrombin-catalyzed hydrolysis of small p-nitroanilide substrates. Similarly, we have tested the ability of a (D-Phe)-Pro-Arg-Pro-Gly pentapeptide to inhibit thrombin catalytic reactivity.

The pentapeptide was synthesized as described in Example 4, using a BOC-glycine-divinylbenzene resin. The pentapeptide was purified and characterized as described in Example 4.

The effects of both Hirulog-8 and this pentapeptide toward thrombin-catalyzed hydrolysis of Spectrozyme TH were studied as described in Example 9, using fixed peptide concentrations of 50 nM or 10 μ M, respectively. Our results show that while nanomolar concentrations of Hirulog-8 can inhibit the thrombin-catalyzed reaction, concentrations of pentapeptide as high as 10 μ M have no significant effect on the thrombin-catalyzed rate. These data show that the CSDM component of the thrombin inhibitors of this invention is, by itself, only a weak inhibitor of thrombin's catalytic function.

EXAMPLE 38

In Vivo Anticoagulant Activity Of Hirulog-8

We determined the in vivo anticoagulant activity of Hirulog-8 following intravenous administration of this peptide into baboons. We used various dosages of Hirulog-8 ranging from 0.002 to 0.2 mg/kg/min. Baboons (male, 10-15 kg) were sedated with ketamine hydrochloride prior to administration of Hirulog-8. Whole blood from treated and control animals was removed from a catheter placed in the femoral vein and collected into 3.8% sodium citrate (9:1; blood:sodium citrate). Plasma was obtained by standard methods and the APTT was recorded by methods described in Example 10. As shown in FIG. 8, Hirulog-8 yielded a dose-dependent increase in the APTT. A 200% increase in the APTT (considered a therapeutic range) was achieved with the lowest Hirulog dose (0.002 mg/kg/min. infusion).

EXAMPLE 39

Inhibition Of Clot-Bound Thrombin By Hirulog-8

It is known that thrombin can bind to a fibrin clot and, once absorbed, continue to cleave additional fibrinogen, resulting in growth of the clot. Clot-bound thrombin has been shown to be resistant to neutralization by the heparin-anti-thrombin III complex [P. J. Hogg et al., "Fibrin Monomer Protects Thrombin From Inactivation By Heparin-Antithrombin III: Implications for Heparin Efficacy", *Proc. Natl. Acad. Sci. USA.* 86, pp. 3619-23 (1989)], but may be inhibited by antithrombin III-independent inhibitors, such as PPACK, hirudin or Sulfo-Tyr₆₃-N-acetyl-hirudin₅₃₋₆₄. Clot-bound thrombin is believed to play a role in thrombus accretion and in rethrombosis following thrombolytic therapy.

We compared the abilities of Hirulog-8 and heparin to inhibit clot-bound thrombin using the method described by J. I. Weitz et al., "Clot-Bound Thrombin Is

Protected from Heparin Inhibition—A Potential Mechanism for Retrombosis After Lytic Therapy", *Blood*, 74, p. 136a, (1989).

A clinically relevant dose of heparin (0.1 U/ml) inhibited fibrinopeptide A (FPA) release catalyzed by soluble thrombin by approximately 70%. However, a similar dose had no effect on FPA release catalyzed by clot-bound thrombin. In contrast, Hirulog-8 had an almost identical inhibitory effect on FPA release catalyzed by either soluble or clot-bound thrombin (FIG. 9).

This study indicated that Hirulog-8, as well as the other thrombin inhibitors of this invention, are more effective than current drugs in blocking thrombus accretion, increasing the rate of thrombolytic reperfusion and preventing rethrombosis following thrombolytic treatment.

EXAMPLE 40

The Effect Of Hirulog-8 On In Vivo
Platelet-Dependent Thrombosis

Because baboons are known to have similar coagulation and hemostatic responses as man, we utilized a baboon model to determine the ability of Hirulog-8 to interrupt platelet-dependent thrombosis. Specifically, we placed various thrombogenic surfaces in a chronic exteriorized AV shunt in the animals. These surfaces included segments of endarterectomized baboon aorta, collagen-coated silastic tubing, collagen-coated Gortex and Dacron vascular graft. Following placement in the shunt, the surfaces were exposed to native flowing blood to elicit thrombus formation. We measured the formation of thrombi over a period of 60 minutes by monitoring the deposition of platelets on the thrombogenic surface. These measurements were recorded by external gamma-camera imaging following pre-infusion of the test animal with autologous ¹¹¹In-labeled platelets.

Placement of a 5 cm segment of endarterectomized baboon aorta in the exteriorized AV shunt in the absence of Hirulog-8 led to a time-dependent deposition of platelets. This accumulation reached a plateau in 60 minutes, at which time a total of $14.0 \pm 5.0 \times 10^8$ platelets/cm were found deposited on the endarterectomized segment. Doses of 0.002 and 0.01 mg/kg/min of Hirulog-8 inhibited platelet deposition by 53.6% and 75.5%, respectively. These results are depicted in FIG. 10. The ED₅₀ for Hirulog-8 (the dosage required to reduce platelet deposition by 50%) in this model system was 0.002 mg/kg/min.

When we placed 5 cm segments of collagen-coated silastic tubing in the AV shunt, $12.6 \pm 5.0 \times 10^8$ platelets/cm were deposited after 60 minutes in the absence of Hirulog-8. Administration of Hirulog-8 resulted in a dose-dependent inhibition of platelet deposition. A dosage of 0.04 mg/kg/min Hirulog-8 completely inhibited platelet deposition. The results of this portion of the experiment are depicted in FIG. 11. The ED₅₀ of Hirulog-8 in this system was calculated to be 0.004 mg/kg/min.

Both collagen-coated Gortex or Dacron vascular grafts are known to be more thrombogenic than silastic tubing. A total of $35.0 \pm 6.0 \times 10^8$ platelets/cm were deposited on the Gortex following a 60 minute exposure to native blood in the absence of Hirulog-8. We found that Hirulog-8 once again demonstrated a dose-dependent antithrombotic effect towards platelet thrombus

formation. A dose of 0.2 mg/kg/min Hirulog-8 caused a 62.9% inhibition of platelet deposition. The ED₅₀ for Hirulog-8 in the Gortex system Was 0.135 mg/kg/min. A similar result was obtained for Dacron grafts. The higher dosage of Hirulog-8 required to inhibit platelet deposition on these two surfaces was to be expected because of their high thrombogenic activity.

We also determined the effect of Hirulog-8 toward both high and low shear platelet-dependent thrombus formation using a dual-chamber device, which allowed for simultaneous measurements of both shear conditions. The device was comprised of a 2 cm segment of collagen-coated Gortex followed by 2 cm segments of expanded diameter. Using this device, thrombus formation was initiated by exposure of native flowing blood to a segment of the collagen-coated Gortex at high shear. This part of the experimental protocol simulated arterial-like conditions. When the blood entered the expanded diameter segments, low-shear, vortex conditions were maintained, thereby simulating venous thrombosis. In control animals, a total of $9.3 \pm 2.3 \times 10^8$ and $6.1 \pm 0.5 \times 10^8$ platelets/cm accumulated after 40 minutes in the high and low shear segments, respectively. Hirulog-8 inhibited platelet deposition in both high and low shear segments in a dose-dependent fashion. A dose of 0.05 mg/kg/min inhibited platelet accumulation by 42.6% at low shear and by 29.0% at high shear.

EXAMPLE 41

Comparison Of Hirulog-8 With Other Anti-Thrombotic Agents In Inhibiting Acute Platelet-Dependent Thrombosis

We examined the effects of heparin, low molecular-weight heparin and recombinant hirudin on platelet deposition in the collagen-coated silastic tubing/externalized AV shunt baboon model described in Example 40.

It has previously been shown that heparin administered as a 160 U/kg bolus injection followed by a 160 U/kg/hr infusion inhibited platelet deposition to a level of about 80% of that observed in a saline-treated control animal. Low molecular-weight heparin, given as a bolus injection of 53 anti-Xa U/kg, followed by infusion at 53 anti-Xa U/kg/hr, yielded similar results [Y. Cadroy, "In Vivo Mechanism of Thrombus Formation. Studies Using a Primate Model", *Doctoral Thesis*, L'Universite Paul Sabatier de Toulouse (Sciences) (1989)]. At equivalent molar doses (5 nmole/kg/min), recombinant hirudin [A. B. Kelly et al., "Recombinant Hirudin Interruption of Platelet-Dependent Thrombus Formation", *Circulation*, 78, p. II-311 (1988)] and Hirulog-8 both inhibited platelet-dependent thrombus formation by 60-70% as compared to the control. These results are depicted in FIG. 12. Other thrombin inhibitors have previously been tested in the baboon model [A. B. Kelley et al., "Comparison of Antithrombotic and Antihemostatic Effects Produced by Antithrombins in Primate Models of Arterial Thrombosis", *Thromb. and Hemostas.*, 62, p. 42 (1989)]. The reported ED₅₀ doses on collagen-coated surfaces for those agents, as well as our ED₅₀ determinations, are summarized in the table below:

Agent	ED ₅₀
PPACK	75 amoles/kg/min
Gyki 14,451	500
Benzamidine	3000

-continued

Agent	ED ₅₀
Argipidine (MD805)	550
rec-Hirudin	<5
Hirulog-8	<5

EXAMPLE 42

The Effect Of Hirulog-8 On Fibrin Deposition

We measured the effect of Hirulog-8 on the deposition of fibrin(ogen) in the thrombi formed in the endarterectomized aortic and collagen-coated silastic tubing segments model systems described in Example 40. Fibrin deposition was determined by measurement of ¹²⁵I-fibrin(ogen) 30 days after completion of the ¹¹¹In-platelet assay described above. This allowed the ¹¹¹In label to decay to a non-interfering level.

FIG. 13 demonstrates that in the absence of Hirulog-8, 0.17 mg/cm fibrin was deposited on the collagen-coated tubing following the 60 minute exposure to flowing blood described in Example 40. Doses of 0.01 and 0.04 mg/kg/min completely inhibited fibrin(ogen) deposition. Similar results were obtained with the endarterectomized aortic segment model. These results show that the thrombin inhibitors of this invention are effective in reducing fibrin(ogen) deposition associated with a thrombus, as well as blocking acute platelet-dependent thrombus formation.

EXAMPLE 43

Measurement Of Clearance Times For Hirulog-8

We used a baboon model to determine Hirulog-8 clearance times following intravenous infusion, single bolus intravenous injection and single bolus subcutaneous injection. APTT assays, performed as described in Example 11, were used to monitor clearance times.

We administered various dosages of Hirulog-8 (0.002-0.2 mg/kg/min) to baboons via systemic intravenous infusion, over a period of 60 minutes. APTT was measured following the 60 minute infusion and at various time intervals thereafter. We determined the average half-time for Hirulog-8 clearance to be 9.2 ± 3.3 minutes.

To determine clearance time after a single bolus injection, we injected baboons with a dose of 1 mg/kg Hirulog-8 intravenously or subcutaneously. APTT measurements were taken at various time intervals following injection. FIG. 14 demonstrates that APTT increased to a peak of 570% of control value 2 minutes after intravenous injection. The half-life of Hirulog-8 following intravenous injection was 14 minutes.

FIG. 15 demonstrates that at the earliest time point following subcutaneous injection of Hirulog-8 (i.e. 15 minutes), APTT was increased to approximately 200% of control. Clearance via the subcutaneous route was prolonged to a half-time of 340 minutes. Hirulog-8 administered subcutaneously was found to be quantitatively adsorbed.

EXAMPLE 44

Effect Of Hirulog-8 In Baboon Models Of Disseminated Intravascular Coagulation

We induced septicemia in baboons by injection of a lethal dose of live *E. coli* according to the method described by F. B. Taylor et al. *J. Clin. Invest.*, 79, pp. 918-25 (1987). Hirulog-8 was infused at a dose of 0.08

mg/kg/hr from 15 minutes prior to the injection of *E. coli* to up to 6 hours following injection. In the absence of Hirulog-8, *E. coli*-induced septic shock led to a marked decline in neutrophil count, blood pressure and hematocrit. Control animals displayed a reduction in hematocrit to 70% of baseline and a drop in blood pressure to 20% of baseline after 3 hours. Administration of Hirulog-8 completely attenuated hematocrit drop and limited the peak drop in blood pressure to 60% of baseline.

Despite attenuation of DIC by Hirulog-8, the lethal infusion of *E. coli* still resulted in morbidity. An autopsy of both control and Hirulog-8-treated animals revealed massive tissue edema in both groups. However, only the control group displayed intravascular thrombosis. The results of the autopsies show that interruption of the coagulopathic stage of septicemia alone is not sufficient to prevent morbidity due to septic shock.

EXAMPLE 45

Effect Of A Combination Of tPA And Hirulog-8 On Thrombolysis

To determine the effect of Hirulog-8 on potentiating tPA-induced thrombolysis, we used a rat model for arterial thrombolysis. In this model, an experimental thrombus was formed in the abdominal aorta following balloon catheter denudation and high grade (95%) stenosis. Blood flow and blood pressure were recorded distal to the site of injury and stenosis. We randomized the rats to received tPA (1.0 mg/kg bolus followed by 1.0 mg/kg/hr infusion) together with one of the following: saline, heparin [10 U/kg bolus, followed by 1.5 U/kg/min infusion), recombinant hirudin (1.0 mg/kg bolus followed by 0.02 mg/kg/hr infusion) or Hirulog-8 (0.6 mg/kg bolus followed by 0.02 mg/kg/hr infusion). The antithrombotic agent or saline was administered concomitant with tPA and for an additional 50 minutes following the end of tPA infusion.

FIG. 16 depicts the results of these experiments. Animals treated with tPA +saline exhibited reperfusion times of 16.2 minutes. Heparin reduced reperfusion time to 12.2 minutes, while recombinant hirudin reduced it to 13.0 minutes. Neither of these decreases were statistically significant ($p < 0.05$). The combination of Hirulog-8 with tPA significantly reduced reperfusion time to 4.4 minutes ($p < 0.01$), thus accelerating the fibrinolytic effect of tPA by a factor of four.

Heparin, hirudin and Hirulog-8 all significantly prevented reocclusion as compared to saline-treated controls (FIG. 17). Each of these agents also prolonged APTT to values of 600%, 300% and 400%, respectively, over control values (FIG. 18). Finally, each of heparin, hirudin and Hirulog-8 increased the time of vessel patency to values of 80.2%, 82% and 93.1%, respectively (control=43.6%) (FIG. 19). These results demonstrate that the thrombin inhibitors of the present invention are superior to other known anti-thrombotics in increasing the efficacy of tPA.

EXAMPLE 46

Effect Of Hirulog-8 And Other Antithrombotic Agents On Bleeding Times In Baboons

We employed the template bleeding time measurement to examine the effects of Hirulog-8 on hemostasis.

Various dosages of Hirulog-8 (0.002 to 0.2 mg/kg/min) were analyzed for their effect on bleeding time. Doses of 0.002 to 0.04 mg/kg/min caused no significant increase in bleeding times. The results of this

experiment are depicted in FIG. 20. At a dose of 0.1 mg/kg/min, Hirulog-8 causes a two-fold increase in bleeding time over control values. At 0.2 mg/kg/min Hirulog-8, bleeding times increased to 3 times control values. These results clearly demonstrate that dosages required to inhibit platelet-dependent thrombosis (0.002 mg/kg/min; see Example 40) do not cause a significant effect on hemostatic plug formation.

We also tested the effects of a variety of other agents on template bleeding time in the baboon, as well as on systemic anticoagulant effects (as measured by APTT). These results are summarized below:

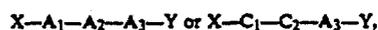
Agent	APTT (% control)	Bleeding Time (min)
Hirulog-8	300.6	5.5
rec-hirudin	393.9	12.1
PPACK	287.9	12
Gyki 14,451	439.4	14
Benzamidine	757.6	10
Argipidine (MD805)	>900	>30
Heparin	706.1	10

While we have hereinbefore presented a number of embodiments of this invention, it is apparent that our basic construction can be altered to provide other embodiments which utilize the molecules, compositions, combinations and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the claims appended hereto rather than the specific embodiments which have been presented hereinbefore by way of example.

We claim:

1. A thrombin inhibitor consisting of:

a) a catalytic site-directed moiety that binds to and inhibits the active site of thrombin; wherein said catalytic site-directed moiety is selected from serine proteinase inhibitors, heterocyclic protease inhibitors, thrombin-specific inhibitors, transition state analogues, benzamidine, DAPA, NAPAP, argipidine, or moieties of the formulae:



wherein X is hydrogen or is characterized by a backbone chain consisting of from 1 to 35 atoms; A₁ is Arg, Lys or Orn; A₂ is a non-amide bond; A₃ is characterized by a backbone chain consisting of from 1 to 9 atoms, Y is a bond; C₁ is a derivative of Arg, Lys or Orn comprising a carboxylate moiety that is reduced, or displaced from the α -carbon by a moiety characterized by a backbone chain consisting of from 1 to 10 atoms; and C₂ is a non-cleavable bond;

b) a linker moiety characterized by a backbone chain having a calculated length of between about 18 Å and about 42 Å; and

c) an anion binding exosite associating moiety; said catalytic site-directed moiety being bound to said linker moiety and said linker moiety being bound to said anion binding exosite associating moiety; wherein said inhibitor is capable of simultaneously binding to the catalytic site and the anion binding exosite of thrombin.

2. The thrombin inhibitor according to claim 1, wherein said anion binding exosite moiety consists of the formula:

W—B₁—B₂—B₃—B₄—B₅—B₆—B₇—B₈—Z;

wherein W is a bond; B₁ is an anionic amino acid; B₂ is any amino acid; B₃ is Ile, Val, Leu, Nle or Phe; B₄ is Pro, Hyp, 3,4-dehydroPro, thiazolidine-4-carboxylate, Sar, any N-methyl amino acid or D-Ala; B₅ is an anionic amino acid; B₆ is an anionic amino acid; B₇ is a lipophilic amino acid selected from the group consisting of Tyr, Trp, Phe, Leu, Nle, Ile, Val, Cha, Pro, or a dipeptide consisting of one of these lipophilic amino acids and any amino acid; B₈ is a bond or a peptide containing from one to five residues of any amino acid; and Z is a carboxy terminal residue selected from OH, C₁—C₆ alkoxy, amino, mono- or di-(C₁—C₄) alkyl substituted amino or benzylamino.

3. The thrombin inhibitor according to claim 2, wherein B₁ is Glu; B₂ is Glu; B₃ is Ile; B₄ is Pro; B₅ is Glu; B₆ is Glu; B₇ is Tyr-Leu, Tyr(SO₃H)-Leu, Tyr(OSO₃H)-Leu or (3-, 5-diiodoTyr)-Leu; B₈ is a bond; and Z is OH.

4. The thrombin inhibitor according to claim 1, wherein said backbone chain of said linker moiety consists of any combination of atoms selected from the group consisting of carbon, nitrogen, sulfur and oxygen.

5. The thrombin inhibitor according to claim 4, wherein said linker comprises the amino acid sequence: Gly-Gly-Gly-Asn-Gly-Asp-Phe.

6. The thrombin inhibitor according to claim 1, wherein said catalytic site-directed moiety binds reversibly to and is slowly cleaved by thrombin.

7. The thrombin inhibitor according to claim 1, wherein said catalytic site-directed moiety binds reversibly to and cannot be cleaved by thrombin.

8. The thrombin inhibitor according to claim 1, wherein said catalytic site-directed moiety binds irreversibly to thrombin.

9. The thrombin inhibitor according to claim 1, wherein X is D-Phe-Pro; A₁ is Arg; and A₃ is D-Pro, Pro, or Sar.

10. The thrombin inhibitor according to claim 9, wherein said thrombin inhibitor is selected from the group consisting of Hirulog-8 and Hirulog-12.

11. The thrombin inhibitor according to claim 1, wherein X is N-acetyl-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val; A₁ is Arg; and A₃ is Pro.

12. The thrombin inhibitor according to claim 1, selected from the group consisting of Hirulog-18a and Hirulog-18b.

13. A pharmaceutically acceptable composition comprising an amount of a thrombin inhibitor according to claim 1, effective for inhibiting a thrombin-mediated function in a patient or in extracorporeal blood and a pharmaceutically acceptable carrier.

14. The pharmaceutically acceptable composition according to claim 13, wherein said pharmaceutically effective amount is between about 1 µg/kg body weight/day to about 5 mg/kg body weight/day.

15. The pharmaceutically acceptable composition according to claim 14, wherein said pharmaceutically effective amount is between about 10 µg/kg body weight/day to about 500 µg/kg body weight/day.

16. A composition for coating the surface of an invasive device to be inserted into a patient, wherein said composition comprises a suitable buffer and at least one thrombin inhibitor according to claim 1.

17. A pharmaceutically acceptable combination for treating or preventing thrombotic disease in a patient comprising:

- a) a thrombin inhibitor according to claim 1;
- b) a thrombolytic agent; and
- c) a pharmaceutically acceptable carrier.

18. The pharmaceutically acceptable combination according to claim 17, wherein said thrombin inhibitor is Hirulog-8 and said thrombolytic agent is tPA.

19. The combination according to claim 17, wherein the daily dosage of said thrombin inhibitor is between about 1 µg/kg body weight and about 5 mg/kg body weight and wherein the daily dosage of said thrombolytic agent is between about 10% and about 80% of the conventional dosage range of said thrombolytic agent.

20. The combination according to claim 19, wherein the daily dosage of said thrombin inhibitor is between about 10 µg/kg body weight and about 500 µg/kg body weight and wherein the daily dosage of said thrombolytic agent is between about 10% and about 70% of the conventional dosage range of said thrombolytic agent.

21. The thrombin inhibitor according to claim 2, wherein said linker moiety is characterized by a backbone chain having a calculated length of between about 18 Å and 36 Å and is selected from the group consisting of an acyl group of from about 17 to 35 carbon atoms, an alkyl group of from about 17 to 35 backbone bonds, a peptide containing from about 6 to 12 residues of any amino acid and combinations thereof.

22. The thrombin inhibitor according to claim 3, wherein:

B₇ is Tyr(SO₃H)-Leu or Tyr(OSO₃H)-Leu; the linker is a peptide of from about 8 to 10 amino acids, the amino acid of said linker which is closest to the anion binding exosite moiety being Phe; and the catalytic site-directed moiety consists of the formula:

X—Arg—R,

wherein X is selected from the group consisting of D-Phe-Pro and tosyl-Gly; and R is selected from group consisting of Pro, Sar and N-methyl Ala.

23. The thrombin inhibitor according to claim 11, wherein said thrombin inhibitor is Hirulog-33.

24. The composition according to any one of claims 13-15 or 16, wherein said thrombin inhibitor is Hirulog-8.

25. The combination according to any one of claims 17, 19 or 20, wherein said thrombin inhibitor is Hirulog-8.

26. A method for decreasing the dose of a thrombolytic agent effective to establish reperfusion or to delay reocclusion in a patient, said method comprising the step of administering said thrombolytic agent to said patient as part of a combination according to claim 18.

27. A method for decreasing the reperfusion time and increasing the reocclusion time in a patient treated with a thrombolytic agent, said method comprising the step of administering to said patient a composition according to claim 13, wherein said composition is administered to said patient during the time period ranging from about 5 hours prior to about 5 hours following the treatment of said patient with said thrombolytic agent.

28. The method according to claim 27, wherein said composition is administered to said patient during the time period ranging from about 2 hours prior to about 2 hours following said treatment of said patient with said thrombolytic agent.

29. A method of inhibiting thrombin's catalytic and receptor-mediated functions in a patient or in extracor-

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poreal blood comprising the step of treating said patient or said extracorporeal blood with a composition according to claim 13.

30. The method according to claim 29, wherein said method is used for treating or preventing a thrombotic disease in a patient.

31. The method according to claim 29, wherein said method is used for treating or preventing thrombin-induced inflammation in a patient.

32. The method according to claim 31, wherein said inflammation is caused by a disease selected from the group consisting of adult respiratory distress syndrome, septic shock, septicemia and reperfusion damage.

33. The method according to claim 29, wherein said method is used to inhibit thrombus accretion in a patient caused by clot-bound thrombin.

34. The method according to claim 29, wherein said method is used for inhibiting platelet-dependent thrombosis in a patient.

35. The method according to claim 29, wherein said method is used for treating or preventing disseminated intravascular coagulation in a patient.

36. The method according to any one of claims 26-28 or 29-35, wherein said patient is a human.

37. The method according to any one of claims 26-28 or 29-35, wherein said thrombin inhibitor employed in said composition or combination is Hirulog-8.

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,196,404 Page 1 of 5
DATED : March 23, 1993
INVENTOR(S) : John M. Maraganore et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

- Col. 2, line 20, "(i985)" should be (1985).
Col. 2, line 59, "th" should be "the".
Col. 3, line 54, "ha" should be -- has --.
Col. 6, line 39, "(C₁-C₄)" should be -- (C₁-C₄).
Col. 8, line 63, "A," should be -- A₃ --.
Col. 9, line 58, "B," should be -- B₃ --.
Col. 10, line 10, "B₇" should be -- B₃ --.
Col. 10, line 25, after "prothrombin", insert
-- fragment --.
Col. 11, line 7, "solution phase" should be
-- solution-phase --.
Col. 17, lines 7, 8, 12, 46, 50, 51, 55 and 57,
"hirudin₅₃₋₆₄" should be -- hirudin₅₄₋₆₄ --.
Col. 17, line 41, "³⁵S]" should be -- [³⁵S] --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,196,404 Page 2 of 5
DATED : March 23, 1993
INVENTOR(S) : John M. Maraganore et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

- Col. 17, line 63, "A," should be -- A₂ --.
- Col. 18, line 3, "C₁" should be -- C₈ --.
- Col. 19, line 26, delete "," after "and".
- Col. 19, line 61, "7-benzyl" should be -- -benzyl --.
- Col. 19, lines 63-64, "BOC-L-phenylalanine" should be -- BOC-D-phenylalanine --.
- Col. 20, line 34, "Arg-L" should be -- Arg-D --.
- Col. 20, line 54, "Hirulog 11" should be -- Hirulog-11 --.
- Col. 20, line 63, "Hirulog 12" should be -- Hirulog-12 --.
- Col. 21, line 61, "K₁" should be -- K_i --.
- Col. 22, lines 16, 17, 44, "K1" should be -- K_i --.
- Col. 22, line 29, "studies" should be -- studied --.
- Col. 22, line 45, "K" should be -- K_i --.
- Col. 23, lines 2 and 3, "K₁" should be -- K_i --.
- Col. 23, line 14, "Hirulog-8, K₁, nM" should be -- Hirulog-8, K_i, nM --.
- Col. 25, line 15, "1 28" should be -- 1-28 --.
- Col. 25, line 22, "i minute" should be -- 1 minute --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,196,404 Page 3 of 5
DATED : March 23, 1993
INVENTOR(S) : John M. Maraganore et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

- Col. 25, line 29, "Bot" should be -- Both --.
- Col. 26, lines 1-68 should be single spaced.
- Col. 26, line 52, "EXAMPLE 2" should be -- EXAMPLE 21 --.
- Col. 27, line 51, "AroinineDiazomethvlketone" should be -- ArginineDiazomethylketone --.
- Col. 28, line 29, "148" should be -- 1.48 --.
- Col. 29, line 1, "NHOH" should be -- NH₄OH --.
- Col. 29, lines 1-68 should be single spaced.
- Col. 29, line 33, "Inc," should be -- Inc., --
- Col. 30, line 68, after "20", insert -- % --.
- Col. 31, in the second line of chemical structures, first structure, "NH" should be -- NH₂ --.
- Col. 31, line 1, "EXAMPLE 23" should be at line 49.
- Col. 33, line 19, "N⁹" should be -- N⁹ --.
- Col. 33, line 20, "Aroininol" should be -- Argininol --.
- Col. 33, line 30, "oaratol-" should be -- paratol- --.
- Col. 35, line 57, "conatining" should be -- containing --.
- Col. 36, line 34, "HPLC₈" should be -- HPLC, --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,196,404 Page 4 of 5
DATED : March 23, 1993
INVENTOR(S) : John M. Maraganore et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 37, line 67, "-Phe-Glu-Glu-Glu-Glu-Ile-" should be
-- -Phe-Glu-Glu-Ile- --.

Col. 39, line 3, "i.45" should be -- 1.45 --.

Col. 40, line 46, "EXAMPLE 29" should be at line 63.

Col. 44, line 34, "concnetrations" should be
-- concentrations --

Col. 44, line 38, "of" should be -- or --.

Col. 44, line 39, "HPL" should be -- HPLC --.

Col. 45, lines 43 and 68, "K₁" should be -- K_i --.

Col. 46, line 31, "K₁" should be -- K_i --.

Col. 47, line 4, "Pentagegtide" should be
-- Pentapeptide --.

Col. 52, line 36, "catalystic" should be -- catalytic --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,196,404

Page 5 of 5

DATED : March 23, 1993

INVENTOR(S) : John M. Maraganore et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 53, lines 6-7, "B₁ is an anionic amino acid" should be
-- B₅ is an anionic amino acid --.

Col. 53, line 11, "form" should be -- from --.

Col. 53, line 17, "B₁ is Glu; B₂ is Ile; B₃ is Pro;" should be
-- B₂ is Glu; B₃ is Ile; B₄ is Pro; --.

Signed and Sealed this

Eighteenth Day of October, 1994

Attest:



BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks



US005196404B1

REEXAMINATION CERTIFICATE (2994th)

United States Patent [19] B1 5,196,404

Marganore et al. [45] Certificate Issued Sep. 10, 1996

[54] INHIBITORS OF THROMBIN

[75] Inventors: John M. Marganore, Concord, Mass.; John W. Fenton, II, Malden Bridge; Toni Kline, New York, both of N. Y.

[73] Assignees: Biogen, Inc., Cambridge, Mass.; Health Research, Inc., Albany, N. Y.

Reexamination Request: No. 90/003,511, Jul. 27, 1994

Reexamination Certificate for: Patent No.: 5,196,404

Issued: Mar. 23, 1993

Appl. No.: 549,388

Filed: Jul. 6, 1990

Certificate of Correction issued Oct. 18, 1994.

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 395,482, Aug. 18, 1989, abandoned.

[51] Int. Cl.⁶ A61K 38/02; A61K 38/00; C07K 7/08; C07K 14/00

[52] U.S. Cl. 514/13; 514/12; 514/14; 530/326; 530/327; 530/325; 530/324; 623/11

[58] Field of Search 530/326, 327, 328, 325, 324; 623/11

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Primary Examiner—Avis M. Davenport

ABSTRACT

[57] This invention relates to novel biologically active molecules which bind to and inhibit thrombin. Specifically, these molecules are characterized by a thrombin anion-binding exosite association moiety (ABEAM); a linker portion of at least 18 Å in length; and a thrombin catalytic site-directed moiety (CSDM). This invention also relates to compositions, combinations and methods which employ these molecules for therapeutic, prophylactic and diagnostic purposes.

B1 5,196,404

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**REEXAMINATION CERTIFICATE
ISSUED UNDER 35 U.S.C. 307**

NO AMENDMENTS HAVE BEEN MADE TO
THE PATENT

2

AS A RESULT OF REEXAMINATION, IT HAS BEEN
DETERMINED THAT:

The patentability of claims 1-37 is confirmed.

* * * * *



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If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. **TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).**

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. **THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.**

ITM NBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY BML YR	ENT	STAT
1	5,196,404	183	990	----	07/549,388	03/23/93	07/06/90	04	NO	PAID

If the "status" column for a patent number listed above does not indicate "PAID" a code or an asterisk (*) will appear in the "status" column. Where an asterisk (*) appears, the codes are set out below by the related item number. An explanation of the codes indicated in the "status" column and as set out below by the related item number appears on the reverse of the maintenance fee statement.

ITM NBR
1
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If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. **THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.**

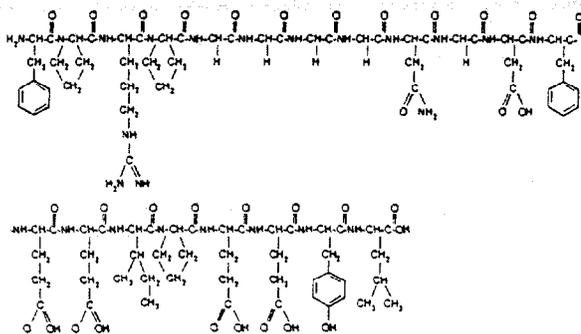
ITEM NBR	PATENT NUMBER	FEE CDE	FEE AMT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY SML YR ENT	STAT
1	5,196,404	184	1900	----	07/549,388	03/23/93	07/06/90	08 NO	PAID

ITM NBR	ATTY DKT NUMBER
1	BI35 CIP

**DIRECT THE RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO:
 COMMISSIONER OF PATENTS AND TRADEMARKS, BOX M. FEE, WASHINGTON, D.C. 20231**

Angiomax™ (bivalirudin) is a specific and reversible direct thrombin inhibitor. The active substance is a synthetic, 20 amino acid peptide. The chemical name is D-phenylalanyl-L-prolyl-L-arginyl-L-prolyl-glycyl-glycyl-glycyl-L-asparagyl-glycyl-L-asparagyl-L-phenylalanyl-L-glutamyl-L-glutamyl-L-isoleucyl-L-prolyl-L-glutamyl-L-glutamyl-L-tyrosyl-L-leucine trifluoroacetate (salt) hydrate (Figure 1). The molecular weight of Angiomax is 2180 daltons (anhydrous free base peptide). Angiomax is supplied in single-use vials as a white lyophilized cake, which is sterile. Each vial contains 250 mg bivalirudin, 125 mg mannitol, and sodium hydroxide to adjust the pH to 5 to 6 (equivalent of approximately 12.5 mg sodium). When reconstituted with Sterile Water for Injection the product yields a clear to opalescent, colorless to slightly yellow solution, pH 5-6.

Figure 1: Structural Formula for Bivalirudin



CLINICAL PHARMACOLOGY

General:

Angiomax directly inhibits thrombin by specifically binding both to the catalytic site and to the anion-binding exosite of circulating and clot-bound thrombin. Thrombin is a serine proteinase that plays a central role in the thrombotic process, acting to cleave fibrinogen into fibrin monomers and to activate Factor XIII to Factor XIIIa, allowing fibrin to develop a covalently cross-linked framework which stabilizes the thrombus; thrombin also activates Factors V and VIII, promoting further thrombin generation, and activates platelets, stimulating aggregation and granule release. The binding of Angiomax to thrombin is reversible as thrombin slowly cleaves the Angiomax-Arg¹-Pro² bond, resulting in recovery of thrombin active site functions.

In *in vitro* studies, bivalirudin inhibited both soluble (free) and clot-bound thrombin, was not neutralized by products of the platelet release reaction, and prolonged the activated partial thromboplastin time (aPTT), thrombin time (TT), and prothrombin time (PT) of normal human plasma in a concentration-dependent manner. The clinical relevance of these findings is unknown.

Pharmacokinetics:

Bivalirudin exhibits linear pharmacokinetics following intravenous (IV) administration to patients undergoing percutaneous transluminal coronary angioplasty (PTCA). In these patients, a mean steady state bivalirudin concentration of 12.3 ± 1.7 mcg/mL is achieved following an IV bolus of 1 mg/kg and a 4-hour 2.5 mg/kg/h IV infusion. Bivalirudin is cleared from plasma by a combination of renal mechanisms and proteolytic cleavage, with a half-life in patients with normal renal function of 25 minutes. The disposition of bivalirudin was studied in PTCA patients with mild and moderate renal impairment and in patients with severe renal impairment. Drug elimination was related to glomerular filtration rate (GFR). Total body clearance was similar for patients with normal renal function and with mild renal impairment (60-89mL/min). Clearance was reduced approximately 20% in patients with moderate and severe renal impairment and was reduced approximately 80% in dialysis-dependent patients. See Table 1 for pharmacokinetic parameters and dose reduction recommendations. For patients with renal impairment the activated clotting time (ACT) should be monitored. Bivalirudin is hemodialyzable. Approximately 25% is cleared by hemodialysis.

Bivalirudin does not bind to plasma proteins (other than thrombin) or to red blood cells.

Table 1. PK parameters and dose adjustments in renal impairment

Renal Function (GFR, mL/min)	Clearance (mL/min/kg)	Half-life (minutes)	% reduction in infusion dose
Normal renal function (≥ 90 mL/min)	3.4	25	0
Mild renal impairment (60-90 mL/min)	3.4	22	0
Moderate renal impairment (30-59 mL/min)	2.7	34	20
Severe renal impairment (10-29 mL/min)	2.8	57	60
Dialysis-dependent patients (off dialysis)	1.0	3.5 hours	90

* The ACT should be monitored in renally-impaired patients

Pharmacodynamics:

In healthy volunteers and patients (with $\geq 70\%$ vessel occlusion undergoing routine angioplasty), bivalirudin exhibits linear dose- and concentration-dependent anticoagulant activity as evidenced by prolongation of the ACT, aPTT, PT, and TT. Intravenous administration of Angiomax produces an immediate anticoagulant effect. Coagulation times return to baseline approximately 1 hour following cessation of Angiomax administration.

In 291 patients with $\geq 70\%$ vessel occlusion undergoing routine angioplasty, a positive correlation was observed between the dose of Angiomax and the proportion of patients achieving ACT values of 300 sec or 350 sec. At an Angiomax dose of 1.0 mg/kg IV bolus plus 2.5 mg/kg/h IV infusion for 4 hours, followed by 0.2 mg/kg/h, all patients reached maximal ACT values > 300 sec.

Clinical Trials:

Angiomax was evaluated in patients with unstable angina undergoing PTCA in 2 randomized, double-blind, multicenter studies with identical protocols. Patients must have had unstable angina defined as: (1) a new onset of severe or accelerated angina or rest pain within the month prior to study entry or (2) angina or ischemic rest pain which developed between four hours and two weeks after an acute myocardial infarction (MI). Overall, 4312 patients with unstable angina, including 741 (17%) patients with post-MI angina, were treated in a 1:1 randomized fashion with Angiomax or heparin. Patients ranged in age from 29-90 (median 63) years, their weight was a median of 80 kg (39-120kg), 68% were male, and 91% were Caucasian. Twenty-three percent of patients were treated with heparin within one hour prior to randomization. All patients were administered aspirin 300-325 mg prior to PTCA and daily thereafter. Patients randomized to Angiomax were started on an intravenous infusion of Angiomax (2.5 mg/kg/h). Within 5 minutes after starting the infusion, and prior to PTCA, a 1 mg/kg loading dose was administered as an intravenous bolus. The infusion was continued for 4 hours, then the infusion was changed under double-blinded conditions to Angiomax (0.2 mg/kg/h) for up to an additional 30 hours (patients received this infusion for an average of 14 hours). The ACT was checked at 5 minutes and at 45 minutes following commencement. If on either occasion the ACT was <350 seconds, an additional double-blinded bolus of placebo was administered. The Angiomax dose was not titrated to ACT. Median ACT values were: ACT in seconds (5th percentile-95th percentile): 345 sec (240-595 seconds) at 5 min and 348 sec (range 269-583 sec) at 45 min after initiation of dosing. Patients randomized to heparin were given a loading dose (175 IU/kg) as an intravenous bolus 5-minutes before the planned procedure, with immediate commencement of an infusion of heparin (15 IU/kg/h). The infusion was continued for 4 hours. After 4-hours of infusion, the heparin infusion was changed under double-blinded conditions to heparin (15 IU/kg/hour) for up to 20 additional hours

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Angiomax™

(bivalirudin)
FOR INJECTION

the ACT was <350 seconds, an additional double-blind bolus of heparin (60 IU/kg) was administered. Once the target ACT was achieved for heparin patients, no further ACT measurements were performed. All ACTs were determined with the Hemochron™ device. The protocol allowed use of open label heparin at the discretion of the investigator after discontinuation of blinded study medication whether or not an endpoint event (procedural failure) had occurred. The use of open-label heparin was similar between Angiomax and heparin treatment groups (about 20% in both groups).

The studies were designed to demonstrate the safety and efficacy of Angiomax in patients undergoing PTCA as a treatment for unstable angina as compared with a control group of similar patients receiving heparin during and up to 24 hours after initiation of PTCA. The primary protocol endpoint was a composite endpoint called procedural failure, which included both clinical and angiographic elements measured during hospitalization. The clinical elements were: the occurrence of death, MI, or urgent revascularization, adjudicated under double-blind conditions. The angiographic elements were: impending or abrupt vessel closure. The protocol-specified safety endpoint was major hemorrhage. The median duration of hospitalization was 4 days for both the Angiomax treatment group and the heparin treatment group. The rates of procedural failure were similar in the Angiomax and heparin treatment groups. Study outcomes are shown in Table 2.

Table 2. Incidences of In-hospital Clinical Endpoints in Randomized Clinical Trials Occurring within 7 Days

	ANGIOMAX™ n=2161	HEPARIN n=2151
Efficacy Endpoints:		
Procedural Failure ¹	7.9%	9.3%
Death, MI, Revascularization	6.2%	7.9%
Death	0.2%	0.2%
MI ²	3.3%	4.2%
Revascularization ³	4.2%	5.6%
Safety Endpoint:		
Major Hemorrhage ⁴	3.5%	9.3%

¹ The protocol specified primary endpoint (a composite of death or MI or clinical deterioration of cardiac origin requiring revascularization or placement of an aortic balloon pump or angiographic evidence of abrupt vessel closure).

² Defined as: Q-wave MI; CK-MB elevation $\geq 2 \times$ ULN, new ST- or T-wave abnormality, and chest pain ≥ 30 mins; OR new LBBB with chest pain ≥ 30 mins and/or elevated CK-MB enzymes; OR elevated CK-MB and new ST- or T-wave abnormality without chest pain; OR elevated CK-MB.

³ Defined as: any revascularization procedure, including angioplasty, CABG, stenting, or placement of an intra-aortic balloon pump.

⁴ Defined as the occurrence of any of the following: intracranial bleeding, retroperitoneal bleeding, clinically overt bleeding with a decrease in hemoglobin ≥ 3 g/dL or leading to a transfusion of ≥ 2 units of blood.

INDICATIONS AND USAGE

Angiomax is indicated for use as an anticoagulant in patients with unstable angina undergoing percutaneous transluminal coronary angioplasty (PTCA). Angiomax is intended for use with aspirin and has been studied only in patients receiving concomitant aspirin (see Clinical Trials and DOSAGE AND ADMINISTRATION).

The safety and effectiveness of Angiomax have not been established when used in conjunction with platelet inhibitors other than aspirin, such as glycoprotein IIb/IIIa inhibitors (see PRECAUTIONS, Drug Interactions).

The safety and effectiveness of Angiomax have not been established in patients with unstable angina who are not undergoing PTCA or in patients with other acute coronary syndromes.

CONTRAINDICATIONS

Angiomax is contraindicated in patients with:

- active major bleeding;
- hypersensitivity to Angiomax or its components.

WARNINGS

Angiomax is not intended for intramuscular administration. Although most bleeding associated with use of Angiomax in PTCA occurs at the site of arterial puncture, hemorrhage can occur at any site. Unexplained fall in blood pressure or hematocrit, or any unexplained symptom, should lead to serious consideration of a hemorrhagic event and cessation of Angiomax administration.

There is no known antidote to Angiomax. Angiomax is hemodialyzable (see CLINICAL PHARMACOLOGY, Pharmacokinetics).

PRECAUTIONS

General:

Clinical trials have provided limited information for use of Angiomax in patients with heparin-induced thrombocytopenia/heparin-induced thrombocytopenia-thrombocytosis syndrome (HIT/HITTS) undergoing PTCA. The number of HIT/HITTS patients treated is inadequate to reliably assess efficacy and safety in these patients undergoing PTCA. Angiomax was administered to a small number of patients with history of HIT/HITTS or active HIT/HITTS and undergoing PTCA in an uncontrolled, open-label study, and in an emergency treatment program and appeared to provide adequate anticoagulation in these patients. In *in-vitro* studies, bivalirudin exhibited no platelet aggregation response against sera from patients with a history of HIT/HITTS.

Drug Interactions:

Bivalirudin does not exhibit binding to plasma proteins (other than thrombin) or red blood cells. Drug-drug interaction studies have been conducted with the adenosine diphosphate (ADP) antagonist ticlopidine, and the glycoprotein IIb/IIIa inhibitor abciximab and with low molecular weight heparin. Although data are limited, precluding conclusions regarding efficacy and safety, in combination with these agents, the results of the studies are consistent with the expected effects of these agents.

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Regulatory Correspondence Log**

Submission Number	Date	To	From	Content
IND 065	2/23/93	Stephen B. Fredd	Michael Slater	New site for the manufacture of Hirulog.
IND 066	3/12/93	Stephen B. Fredd	Michael Slater	Protocol Amendment: C92-304-P "A Multicenter, Double-Blind, Randomized Study to Compare the Safety and Efficacy of BG8967 with Heparin in Patients with Unstable Angina Undergoing Percutaneous Transluminal Coronary Angioplasty (PTCA)" Incorporation of FDA recommendations.
IND 067	4/1/93	Stephen B. Fredd	Michael Slater	Follow-up IND Safety Report: C92-301-P "A Multicenter, Double-Blind, Randomized Study to Compare Four Doses of BG8967 in Patients with Unstable" (Patient Died).
IND 068	4/16/93	FDA	Michael Slater	Information received during the months of January, February, and March: Protocol Amendment (New Investigator); Other (Compassionate Use).
IND 069	5/3/93	Stephen B. Fredd	Michael Slater	Meeting request: CMC
IND 070	5/14/93	Stephen B. Fredd	Michael Slater	New vial and stopper
IND 071	5/24/93	Stephen B. Fredd	Michael Slater	Revised plans to use BG8967 as a continuous intravenous infusion over a range of four hours to 3-5 days in the treatment of patients undergoing PTCA or suffering from unstable angina.
IND 072	6/4/93	Stephen B. Fredd	Michael Slater	Response to FDA request of June 2 for information regarding CMC: solid phase synthesis; pilot scale solution phase synthesis; commercial scale solution phase synthesis.
IND 073	6/7/93	Stephen B. Fredd	Michael Slater	Attendee list for June meeting regarding manufacturing.
IND 074	6/25/93	FDA	Michael Slater	Information received during the months of April, May and June 1993
IND 075	7/8/93	Stephen B. Fredd	Michael Slater	Study C92-304-P; patient needs to undergo an atherectomy procedure
IND 076	7/14/93	Stephen B. Fredd	Michael Slater Burt Adelman	New Protocol C92-307-P "A Randomized, Double-Blind Comparison of Hirulog (BG8967) Plus Streptokinase versus Intravenous Heparin Plus Streptokinase in Suspected Acute Myocardial Infarction.
IND 077	8/6/93	Stephen B. Fredd	Michael Slater	Eight copies of submission #76 for the August 19, 1993 meeting.
IND 078	8/9/93	Stephen B. Fredd	Michael Slater	AE (ADR 93/01/BG8967) in study C92-304-P; groin hematoma leading to left occipital bleed, increase in bleed and ultimate left craniotomy.
IND 079	8/17/93	Stephen B. Fredd	Michael Slater	AE (ADR 93/02/BG8967) in study C92-304-P "impending closure"
IND 080	8/20/93	Stephen B. Fredd	Michael Slater	Alternative Manufacturing Site
IND 081	8/23/93	Stephen B. Fredd	Michael Slater	Request for FDA meeting minutes (7 meetings from December 89 through August 93)
IND 082	9/2/93	FDA	Michael Slater	Information received during the months of July and August 1993; Protocol Amendment (New Investigator); Information Amendment (Clinical)
IND 083	9/9/93	Stephen B. Fredd	Michael Slater	New Protocol C93-312-P entitled "Use of BG8967 as an Anticoagulant and Antithrombotic in Patients with Heparin-Associated Thrombocytopenia (HAT)"
IND 084	9/9/93	Stephen B. Fredd	Michael Slater	Authorize Christopher Cannon, MD of Brigham & Women's Hospital, Boston to reference IND 35,756.
IND 085	9/30/93	Stephen B. Fredd	Michael Slater	New Protocol C93-313-P entitled "A Pharmacokinetic and Pharmacodynamic Study of BG8967 in Subjects with Renal Insufficiency"
	10/5/93	Michael Slater	Stephen B. Fredd	Meetings minutes from December 14, 1989, March 28, 1991, February 5, 1992, August 5, 1992, November 23, 1992 and June 9, 1993
IND 086	10/13/93	Stephen B. Fredd	Michael Slater	Follow-up safety report ADR93/01/BG8967 and ADR 93/02/BG8967 from study C92-304-P
IND 087	10/22/93	Stephen B. Fredd	Michael Slater	Request for meeting, scale-up of commercial CMC process.
IND 088	11/3/93	FDA	Michael Slater	Information received during the months of September and October 1993; Protocol Amendment (Change in Protocol); Information Amendment (CMC)
IND 089				Changed to IND submission #090.
IND 090	11/19/93	Stephen B. Fredd	Michael Slater	Protocol Amendment C92-304-P entitled "A Multicenter, Double-Blind, Randomized Study to Compare the Safety and Efficacy of BG8967 with Heparin in Patients with Unstable Angina Undergoing Percutaneous Transluminal Coronary Angioplasty (PTCA)"

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Submission Number	Date	To	From	Content
IND 091	11/24/93	Stephen B. Fredd	Michael Slater	New lyophilized formulation for BG8967, an alternative site for formulation and filling and information on a lyophilized placebo for BG8967.
IND 092	11/24/93	Stephen B. Fredd	Michael Slater Burt Adelman	Change in protocol C92-307-P entitled "A Randomized, Double-Blind Comparison of Hirulog Plus Streptokinase vs. Intravenous Heparin Plus Streptokinase in Suspected Acute Myocardial Infarction" (Version 3)
IND 093	12/2/93	Stephen B. Fredd	Michael Slater	Response to FDA request for information regarding study C93-310-P entitled "A Double-Blind, Randomized Placebo-Controlled Study to Determine the Tolerability and to Compare the Pharmacokinetic Profiles of Two Formulations of BG8967 in Healthy Volunteers". SAS Output
IND 094	12/7/93	Stephen B. Fredd	Michael Slater	Meeting request x 3 for 1) pre-clinical results to-date; 2) environmental assessment for NDA; 3) manufacturing plans at UCB Bioproducts
IND 095	12/7/93	Stephen B. Fredd	Michael Slater	Response to FDA regarding randomization for study C92-304-P entitled "A Multicenter, Double-Blind, Randomized Study to Compare the Safety and Efficacy of BG8967 with Heparin in Patients Undergoing Percutaneous Transluminal Coronary Angioplasty (PTCA)"
IND 096	12/23/93	Stephen B. Fredd	Michael Slater Burt Adelman	Results of study C92-301-P, a blinded, randomized, dose-ranging study to evaluate the efficacy and tolerability of four doses of BG8967 in treatment of patients with unstable angina. Plans to conduct follow-up study C93-309-P.
IND 097	1/26/94	Stephen B. Fredd	Michael Slater	Safety Report (ADR 94/01/BG8967) in study C93-312-P; extensive bleeding
IND 098	2/4/94	Stephen B. Fredd	Michael Slater	1993 Annual Report
IND 099	2/11/94	Stephen B. Fredd	Michael Slater	Request for Meeting Minutes from November 16, 1993 and February 4, 1994
IND 100	2/14/94	Stephen B. Fredd	Michael Slater	Response to FDA request for information regarding preclinical development
IND 101	2/24/94	Stephen B. Fredd	Michael Slater	Safety Report (ADR 94/02/BG8967) from study C92-304-P, decreased urinary output - ultimately renal failure
IND 103	3/4/94	Stephen B. Fredd	Michael Slater	Response to FDA request for information regarding protocol C93-313-P.
IND 104	3/14/94	Stephen B. Fredd	Michael Slater	Information on a revision to bulk and finished product release specifications and test methodology.
IND 105	3/14/94	Stephen B. Fredd	Michael Slater	Meeting information: to discuss how FDA would like the safety and effectiveness data from clinical trials of Hirulog in patients undergoing angioplasty presented.
IND 106	3/17/94	Stephen B. Fredd	Michael Slater	Plans to develop a lyophilized formulation to replace the frozen formulation.
IND 107	3/18/94	Stephen B. Fredd	Michael Slater	Revised ISS and ISE tables for discussion at the March 24 meeting
IND 108	3/23/94	Stephen B. Fredd	Michael Slater	Follow-up Safety Report (ADR 94/01/BG8967) in study C93-312-P.
IND 109	3/25/94	FDA	Michael Slater	Information received during the months of September 1993 through February 1994; Protocol Amendment (New Protocol); Protocol Amendment (New Investigator)
IND 110	4/14/94	Stephen B. Fredd	Michael Slater Burt Adelman	Update on the bleeding complication rates in Hirulog trials
IND 111	4/14/94	Stephen B. Fredd	Michael Slater	Safety Update (ADR 94/03/BG8967) in study C92-304-P; seizure
IND 112	4/21/94	Stephen B. Fredd	Michael Slater	Follow-up to March 7 meeting: quality of processed water used in bulk drug substance manufacturing at UCB-Bioproducts.
IND 113	4/21/94	Stephen B. Fredd	Michael Slater	Request for FDA meeting minutes of March 7, 1994 and March 24, 1994
IND 114	4/27/94	Stephen B. Fredd	Michael Slater	DSMB for clinical trials in angioplasty (Studies C92-304-1, -2) requested unblind data. Results attached
IND 115	5/2/94	FDA	Michael Slater	Information received during the month of April 1994
IND 116	5/27/94	Stephen B. Fredd	Michael Slater Burt Adelman	Update FDA on status of unstable angina program
IND 117	6/10/94	Stephen B. Fredd	Michael Slater	Case report form for protocol C93-309-P entitled "An International, Multicenter, Double-Blind, Randomized Trial to Compare
IND 118	6/20/94	Bronwyn Collier	Stacie L. Pallotta	Resubmitting Appendix I and II from Submission Number 112 dated April 21, 1994 regarding processed water used in bulk drug substance manufacturing at UCB-Bioproducts
IND 119	6/22/94	FDA	Michael Slater	Information received during the month of May 1994.

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Submission Number	Date	To	From	Content
IND 120	7/6/94	Stephen B. Fredd	Michael Slater	Revision to the bulk product release specifications and test methodology for BG8967: The Organic Volatile Impurity assay.
IND 121	7/6/94	Stephen B. Fredd	Michael Slater	Pre-clinical unexpected death of a cynomolgus monkey following infusion of BG8967 at a dose of 6.25 mg/kg/hour for four
	7/11/94	Michael Slater	Steven B. Fredd	Memorandum of Meeting dated March 24, 1994.
IND 122	7/26/94	FDA	Michael Slater	Information received during the months of June and July 1994
IND 123	7/29/94	FDA	Michael Slater	Information received during the months of June and July 1994 (repeat of above); Protocol Amendment (New Investigator);
IND 124	8/1/94	Stephen B. Fredd	Michael Slater	Follow-up Safety Reports ADR 94/02/BG8967 and ADR 94/03/BG8967 in study C92-304-P.
IND 125	8/19/94	FDA	Michael Slater	Information received during the month of August 1994; Protocol Amendment (New Investigator); Information Amendment
IND 126	8/26/94	FDA	Michael Slater	Information received during the month of August 1994 (repeat of above).
IND 127	8/26/94	Stephen B. Fredd	Michael Slater Burt Adelman	Meeting request to discuss the clinical pharmacology section of the Hirulog NDA application
IND 128	9/2/94	Stephen B. Fredd	Michael Slater	Response to FDA request of June 14, 1994 regarding the assay for BG8967 in plasma
IND 129	9/8/94	Stephen B. Fredd	Michael Slater	Safety information (ADR 94/04/BG8967) in study C92-307-P; acute cardiac tamponade.
IND 130	9/14/94	Stephen B. Fredd	Michael Slater	Request for meeting to discuss the scale-up process for the manufacture of bulk drug substance
IND 131	9/21/94	FDA	Michael Slater	Information received during the month of September 1994; Protocol Amendment (New Investigator)
IND 132	9/26/94	Stephen B. Fredd	Michael Slater	Information received during the month of September 1994; Protocol Amendment (New Investigator); Information Amendment (Clinical)
IND 133	9/28/94	Stephen B. Fredd	Michael Slater	Follow-up Safety Report (ADR 94/04/BG8967) in study C92-307-P
IND 134	10/3/94	Stephen B. Fredd	Michael Slater	Background package for the Clinical Pharmacology meeting to be held on October 17, 1994
IND 135	10/14/94	Stephen B. Fredd	Michael Slater	Safety Report (ADR 94-0001/BG8967-309) in study C93-309-P; seizure
IND 136	10/20/94	Stephen B. Fredd	Michael Slater	Safety: three tables of results from studies C92-304-1 & -2
IND 137	10/27/94	FDA	Michael Slater	Information received during the month of September 1994
IND 138	11/2/94	Stephen B. Fredd	Michael Slater	Biogen has decided not to proceed to NDA filing
IND 139	11/3/94	Stephen B. Fredd	Michael Slater	Request FDA meeting minutes of October 17 & 19, 1994
IND 140	12/12/94	FDA	Michael Slater	Information received during the months of October and November 1994. Protocol Amendment (New Investigator); Information Amendment (Clinical).
IND 141	12/29/94	Stephen B. Fredd	Michael Slater	Scale-up process to manufacture bulk drug substance for Hirulog Injection. (formulation of the final lyophilized dosage form remains unchanged, identical to that submitted on November 24, 1993 as serial number 91
IND 142	2/3/95	Stephen B. Fredd	Michael Slater	1994 Annual Report
IND 143	6/19/95	Stephen B. Fredd	Burt Adelman	Burt Adelman, MD, VP RA replaces Irvin D Smith as Sponsor's Authorized Representative
IND 144	8/17/95	Stephen B. Fredd	Burt Adelman	Clinical information pertinent to study C92-307-P entitled "A Randomized, Double-Blind Comparison of Hirulog plus Streptokinase vs. Intravenous Heparin Plus Streptokinase in Suspected Acute Myocardial Infarction
IND 145	10/4/95	Stephen B. Fredd	Burt Adelman	Follow-up to Teleconference regarding adverse event ADR 94/01/BG8967 in study C92-307-P
IND 146	2/2/96	Stephen B. Fredd	Burt Adelman	1995 Annual Report
IND 147	2/19/97	Stephen B. Fredd	Burt Adelman	Changed to 148
IND 148	2/19/97	Stephen B. Fredd	Burt Adelman	1996 Annual Report
IND 149	3/5/97	Stephen B. Fredd	Burt Adelman	Biogen's intent to complete licensing agreement with a partner who will undertake further commercial development
IND 150	---	---	---	This serial number seems to have been skipped
IND 151	---	---	---	This serial number seems to have been skipped
	4/18/97	Thomas Lategan	Julieann DuBeau	Required documentation to complete transfer of IND to TMC
IND 152	5/15/97	FDA	Thomas Lategan	Response to FDA request of April 18, 1997 for information regarding The Medicines Company
	6/4/97	Julie DuBeau	Barbara Finn	Pre-NDA meeting / Clarification on Word Processing

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Submission Number	Date	To	From	Content
IND 153	6/13/97	Lilia Talarico	Barbara Finn	Clinical Amendment: Protocol review of a mortality trial to demonstrate the efficacy and safety of Hirulog of ruse as an anticoagulant with aspirin and streptokinase in acute myocardial infarction.
	6/18/97	Julie DuBeau	Barbara Finn	Case Report Tabulations / Clarification on Division
	6/24/97	Julie DuBeau	Barbara Finn	The AMI protocol submission
IND 154	6/27/97	Julie DuBeau	Barbara Finn	Request for pre-NDA meeting (information not located in file room; unless combined with 155 below for one submission and serial number 154 deemed obsolete).
IND 155	6/27/97	Lilia Talarico	Barbara Finn	Request for pre-NDA meeting and additional information.
	7/7/97	Barbara Finn	Julie DuBeau	The AMI protocol submission / pre-NDA meeting request
IND 156	7/18/97	Lilia Talarico	Barbara Finn	Background package for pre-NDA meeting to be held in October
	7/29/97	Barbara Finn	Lilia Talarico	Completed review of the June 13, 1997 submission #154 of the Hirulog Early Reperfusion/Occlusion Trial (HERO-2)
	7/29/97	Clive Meanwell	M. Kathleen Locke	TMCs request for reduction and deferral of the fiscal year 1997 application fee
	8/8/97	Julie DuBeau	Barbara Finn	Follow-up on pre-NDA meeting
IND 157	8/21/97	Lilia Talarico	Barbara Finn	Proposal for submission of a Segment III study with Hirulog
	8/28/97	Quintiles	Lilia Talarico	Meeting minutes from August 4, 1997
IND 158	9/5/97	Lilia Talarico	Barbara Finn	Sponsor pre-NDA meeting minutes of August 4, 1997
IND 159	9/5/97	Lilia Talarico	Barbara Finn	Response to FDA request of July 29, 1997 regarding amendment 154 containing the HERO-2 protocol
	9/15/97	Barbara Finn	Julie DuBeau	Segment III study and Response to AMI protocol comments (Amendments 147 and 159)
IND 160	9/18/97	Lilia Talarico	Barbara Finn	Request for CMC pre-NDA meeting
	10/7/97	Julie DuBeau	Cynthia Cowthran	Request for a CMC pre-NDA meeting (Amendment 160)
	10/16/97	Ian Fier	Chris Granger	FDA discussion regarding AE reporting
	10/16/97	Ian Fier	Chris Granger	FDA discussion regarding AE reporting
	10/17/97	Barbara Finn	Julie DuBeau	Discussion on SAE reporting issues
IND 161	10/20/97	Lilia Talarico	Barbara Finn	Response to FDA request of July 29 and October 17, 1997 for information in regard to the instruction, capture and resultant analysis of adverse events in the HERO-1 study.
IND 162	10/22/97	Lilia Talarico	Barbara Finn	Preparation of the final lyophilized dosage form and the specifications and controls for Hirulog
IND 163	11/5/97	Lilia Talarico	Barbara Finn	Protocol amendment entitled "The Effect of Hirulog in Combination with Reopro on Laboratory Coagulation Parameters and the Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"
IND 164	11/6/97	Lilia Talarico	Barbara Finn	Sponsor meeting minutes of CMC pre-NDA meeting of October 27, 1997
	11/13/97	Quintiles	Eric P. Duffy	FDA meeting minutes from the October 28, 1997 meeting
IND 165	11/18/97	Lilia Talarico	Barbara Finn	Updated Investigators Brochure
	12/19/97	Quintiles	Eric P. Duffy	CMC submission #162 dated October 22, 1997
NDA 000	12/23/97	Lilia Talarico	Clive Meanwell	Original NDA Submission
	12/29/97	Nathalie DuBois UCB-Bioproducts	Paul Chapman	Receipt of BMF File #12797
IND 166	1/6/98	Lilia Talarico	Barbara Finn	Protocol Amendment entitled The Effect of Hirulog in Combination with Reopro on Laboratory Coagulation Parameters and the Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA
	1/15/98	Quintiles	Julieann DuBeau	Notice that FDA received NDA 20-873 for Hirulog

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Submission Number	Date	To	From	Content
	1/20/98	Barbara Finn	Julie DuBeau	Info to be sent to Division of Scientific Investigations as attached as we discussed today.
IND 167	1/27/98	Lilia Talarico	Barbara Finn	New Protocol entitled "A Multicenter Open-label, Randomized Trial Comparing Clinical Outcome with Hirulog and Provisional Abciximab versus Planned Abciximab and Low-Dose Heparin in Patients Undergoing Percutaneous Intervention.
IND 168	1/27/98	Lilia Talarico	Barbara Finn	Response to FDA Letter of December 19, 1997 requesting clarification regarding amendment 162 dated October 22, 1997 providing some revisions to the QC testing for Hirulog
NDA 001	1/31/98	FDA	Barbara Finn	Provision of CMC Stability Data in SAS and BioPharmaceutics Data in ASCII
IND 169	2/9/98	Lilia Talarico	Barbara Finn	Formal submission of the HERO-2 protocol
NDA 002	2/10/98	FDA	Barbara Finn	Response to FDA request of January 31, 1998 from the Medical Reviewer requesting a print out of enrollment and dropout by study by site; a summary of primary efficacy data by study and by site for C92-304-1 and -2.
	2/17/98	Quintiles	Lilia Talarico	Request information to enable FDA to complete their review of NDA 20-873
IND 170	2/20/98	Lilia Talarico	Barbara Finn	Request for CMC meeting to discuss proposed changes in the drug substance specifications previously presented in amendment 141
NDA 003	2/25/98	FDA	Barbara Finn	Response to FDA letter dated February 11, 1998: Revised, detailed index for the clinical technical section as well as Case Report Tabulations; Proposed annotated labeling on diskette in Word Perfect 6.1; Demographic and efficacy data for the two pivotal studies C92-304-1 and C92-304-2 in Paradox 5.0 for Windows data sets; Statistical analysis of the stability data, including expiration date calculation; or, alternatively, a justification for not conducting the analysis; Delineation of which stability reports are used to establish expiry and which are supportive; AUC values calculated from time zero to the time of the last detectable bivalirudin plasma concentration AUC (0-t) instead of AUC (0-28). Verify that the clearance was calculated as the ratio of dose to AUC (0-∞). Please provide recalculated clearance values for study C93-310; Clarification as to whether the reference made to an <i>in vitro</i> metabolism study in which rat hepatocytes were used for assessment of potential drug-drug interactions with P450 isoenzymes is based on the P8967-92-09 study report. If not, please provide the study report number
	3/12/98	Barbara Finn et al	Julie DuBeau et al	Chromogenic Assay Test Used to Measure Thrombin Inhibition
	3/17/98	Barbara Finn	Lilia Talarico	Biopharmaceutics information
IND 171	3/20/98	E. Duffy A. Shaw	Barbara Finn	Chromogenic assay, TMC agrees with FDA that the chromogenic assay should be considered as an assay indicative of biological potency and the need for this assay to be improved and validated
NDA 004	3/24/98	FDA	Barbara Finn	Response to FDA letters dated February 11 and March 17, 1998 requesting Clinical Study Protocols, amendments and appendices on diskette; BioPharmaceutics: Information for each validation study as follows: assay performance before and during sample analysis, preparation and performance of quality controls, raw data (including data utilized to construct the calibration curves). Stability of bivalirudin in samples, freeze-thaw stability, sample storage conditions, and assay validation. If one validation report supports multiple studies, please identify the validation report that supports each study; Tabulated summary listing assay method, validated analytical range, the dates of assay validation, and sample analysis for each Hirulog study; The assessment of possible contributing factors of period effect and period and treatment interaction for Study 93-310. From March 17 letter: Specifically in regard to item D5, the bioequivalence assessment for C93-310 needs to be repeated with the recalculated AUC values [AUC(0-last) and AUC 0-info]. In addition, please submit as ASCII file on a separate
	4/1/98	Barbara Finn	Lilia Talarico	Proposed proprietary name, Hirulog, is unacceptable.
IND 172	4/2/98	E. Duffy A. Shaw	Barbara Finn	Update FDA on ongoing efforts to evaluate the current chromogenic assay and to propose necessary modifications prior to the validation
	4/6/98	Barbara Finn	Eric P. Duffy	Minutes from the March 12, 1998 teleconference
	4/14/98	Lawrence Parker	Henry L. Fielden	Summary of FDA's inspection of BVL

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Submission Number	Date	To	From	Content
NDA 005	4/24/98	FDA	Barbara Finn	Final Report for Segment III Repro Study
IND 173	5/1/98	E. Duffy A. Shaw	Barbara Finn	Request for CMC meeting to discuss the chromogenic assay plan for modification and validation.
NDA 006	5/1/98	FDA	Barbara Finn	Response to FDA request of April 27, 1998 regarding method of preparation of Hirulog in non-clinical GLP studies
NDA 007	5/11/98	FDA	Barbara Finn	Response to Biopharm reviewers request for information
	5/19/98	Barbara Finn	Lilia Talarico	Request additional information – Biopharmaceutics section
IND 174	5/21/98	E. Duffy A. Shaw	Barbara Finn	Request for meeting of 05/01 is not warranted, assay development report and validation report including the resulting testing methodology SOP for the thrombin inhibition assay is enclosed
IND 175	5/28/98	E. Duffy A. Shaw	Barbara Finn	Supply missing table from the thrombin inhibition assay
	6/12/98	Barbara Finn	Eric P. Duffy	Suggest recommendations / requests regarding the thrombin inhibition assay
	6/15/98	Barbara Finn	Lilia Talarico	Request additional information to continue the Clinical Pharmacology and Biopharmaceutics review
	6/19/98	Barbara Finn	Eric P. Duffy	Request additional CMC information to continue review
	6/24/98	H. Giersiefen J. Richards T. Wright	Barbara Finn	Request TMC assist in providing CMC information to FDA (see above letter from FDA)
	7/6/98	Barbara Finn	Julie DuBeau	Additional Request from Chemistry Reviewer
NDA 008	7/26/98	FDA	Barbara Finn	Response to FDA letter of June 19, 1998 and Telephone Message of July 6, 1998 regarding CMC. Master Batch Record & completed batch record (in English for lots of Hirulog manufactured by Biogen for the pivotal clinical trials; Any new formulations and manufacturing batch records from Ben Venue Laboratories; The measures taken at BVL to ensure that the drug product maintains its potency during the manufacturing process; A description of the validation of the endotoxin assay; On page 100 of Volume 1.003, it is stated that final analytical methods from BVL will be forwarded when they are available. Please update the final methods and provide the validation.
	8/5/98	Fred M. Lockner	Thomas Wright	Proposed sampling plan and acceptance criteria for the statistical analysis of content uniformity on BVL lot numbers 41692, 41693 and 42376.
IND 176	8/11/98	Lilia Talarico	Barbara Finn	Formal Copy of Facsimile to Dr. Shaw
IND 177	8/13/00	Lilia Talarico	Barbara Finn	Revised sampling plan.
	8/14/98	Barbara Finn	?	Recommendations regarding sampling plan for uniformity on BVL
	8/18/98	Barbara Finn	?	Additional recommendations/requests regarding the thrombin inhibition assay
NDA 009	8/20/98	FDA	Barbara Finn	Response to FDA letter dated June 19, 1998 regarding CMC. Additional clarification regarding the master batch record and completed batch record from Amendment #8; Identification of the site that will perform the chromogenic assay for thrombin inhibition on the drug substance and drug product; Hard copy of the facsimile transmission of June 24, 1998 in regard to the pharmacology reviewer's request.
	9/14/98	Barbara Finn	?	Notification that the IND annual report has not been received
IND 178	9/17/98	Lilia Talarico	Barbara Finn	Results of the chromogenic testing based on the revised SOP
	9/28/98	Barbara Finn	Eric P. Duffy	Recommendations/requests regarding results of the chromogenic testing
IND 179	9/29/98	Lilia Talarico	Barbara Finn	Response to FDA request of September 28, 1998 and amendments 172, 174, 175
IND 180	9/29/98	Lilia Talarico	Barbara Finn	Changed to 181
IND 181				CMC Amendment regarding IND amendments 172, 174, 175, 176, 177, 179, 179 and FDA letter dated September 28, 1998.

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Submission Number	Date	To	From	Content
	10/1/98	Lilia Talarico	Barbara Finn	Advisory Committee Briefing Package (October 23, 1998)
IND 182	10/6/98	Lilia Talarico	Barbara Finn	Response to FDA request of October 2, 1998 (telephone) regarding amendments 180/181
NDA 010	10/6/98	FDA	Barbara Finn	Response to October 2, 1998 FDA Telephone Request regarding CMC. A copy of the developmental manufacturing process validation for Hirulog prepared by BVL; Identification and clarification of the site that will perform the chromogenic assay for thrombin inhibition on the drug substance and drug product and a copy of the procedure for this assay; Address of the testing facility for the drug substance and drug product is BioReliance.
	10/8/98	Edna M. Morgan	Thomas Lategan	List of Investigators who have been involved in bivalirudin clinical trials.
	10/15/98	Julie DuBeau	Barbara Finn	Status of Clinical Supplies Release / Misc. NDA Items (Recent amendment #180/181)
	10/16/98	Julie DuBeau	Barbara Finn	Clinical Supplies Release Meeting / Misc. NDA Items
NDA 011	10/19/98	FDA	Barbara Finn	Response to FDA letters dated February 11, March 17, May 19 and June 15, 1998 regarding BioPharmaceutics questions. Information for each validation study as follows: assay performance before and during sample analysis, preparation and performance of quality controls, raw data, stability of Bivalirudin in samples, freeze thaw stability, sample storage conditions, and assay validation. If one validation report supports multiple studies, please identify the validation report that supports each study; Tabulated summary listing assay method, validated analytical range, the dates of assay validation, and sample analysis for each Hirulog study; Information on the methods and equipment used for aPTT measurements by study and the site where the measurements were made as well as how the equipment was calibrated; AUC values calculated from time zero to the time of last detectable Bivalirudin plasma concentration AUC(0-t) instead of AUC (0-28). Verify that clearance was calculated as the ratio of dose to AUC(0-inf). Please provide recalculated clearance values for study C93-310; Your assessment of possible
IND 183	10/20/98	Lilia Talarico	Barbara Finn	Final protocol entitled "The Effect of Hirulog in Combination with Reopro on Laboratory Coagulation Parameters and the Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"
	10/26/98	Barbara Finn	Eric P. Duffy	Recommendations/requests regarding proposed sampling plan
IND 184	10/29/98	Lilia Talarico	John D. Richards	Partial response to FDA letter of October 27, 1998
IND 185	11/3/98	Lilia Talarico	Clive Meanwell	Request for meeting to discuss the future of bivalirudin and steps to move it toward approval.
NDA 012	11/3/98	Julieann DuBeau	Tom Lategan	120 Day Safety Update
	11/5/98	FDA	TMC	Gain an understanding of the process by which we will respond to the October 28 approvable letter
IND 186	11/9/98	Lilia Talarico	John D. Richards	Response to FDA letter of October 27, 1998
IND 187	11/9/98	Lilia Talarico	Tom Lategan	Protocol amendment entitled, "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation Parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA" (Version 3)
	11/15/98	Julie DuBeau	Phyllis Collins	Additional request to TMC response to approvable letter.
	11/16/98	Tom Lategan	Lilia Talarico	Recommendations regarding IND submissions dated September 18, October 29, and November 9, 1998, serial numbers 180, 184, and 186
	11/18/98	Tom Lategan	Paula Botstein	Not Approvable Letter
NDA 013	11/19/98	Paula Botstein	Tom Lategan	Intention to Amend Application in response to FDA Not Approvable Letter dated Nov 18, 1998
NDA 014	11/20/98	Lilia Talarico	Clive Meanwell	Meeting Request: Objective: To agree the most efficient further activities required to enable approval and ensure strong data support for proposed labeling.
IND 188	12/2/98	Lilia Talarico	Tom Lategan	Protocol Amendment (New) entitled "The influence of dose and kidney function on bivalirudin pharmacokinetics and pharmacodynamics in patients undergoing Percutaneous coronary artery angioplasty (PTCA)
IND 189	12/7/98	Lilia Talarico	Tom Lategan	Response to FDA request of December 7, 1998 (telephone) regarding amendment 167 containing the protocol entitled "A Multicenter, Open-Label, Randomized Trial Comparing Clinical Outcome with Hirulog and Provisional Abciximab vs. Planned Abciximab and Low Dose Heparin in Patients Undergoing Percutaneous Intervention

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Submission Number	Date	To	From	Content
	12/7/98	Tom Lategan	Julieann DuBeau	Meeting is scheduled fro January 15, 1999 from 3-4:30 (participants are) regarding protocol entitled "A Multicenter, Open-Label, Randomized Trial Comparing Clinical Outcome with Hirulog and Provisional Abciximab vs. Planned Abciximab and Low Heparin in Patients Undergoing Percutaneous Intervention"
IND 190				Not in file.
IND 191	12/15/98	Lilia Talarico	Barbara Finn	Investigator information for HERO-2 (Frey; Colgate; de Guia; Lefkovic; Nair; Strony; Nallasvian; Stomel; Aylward; Cross; Hamer; Hart; Heddle; Horowitz; Lane; Lim; Logan; Rankin; Simmonds; Soward; Tan; Taylor; Waites; White)
IND 192	12/15/98	Lilia Talarico	John D. Richards	Results of chromogenic testing on lot 42376
NDA 015	12/16/98	Lilia Talarico	Clive Meanwell	Briefing Package for January 15, 1999 meeting (meeting request amendment #14)
IND 193	12/22/98	Lilia Talarico	Barbara Finn	Follow-up Safety Report originally submitted as amendment 169; drug hypersensitivity reaction
IND 194	12/22/98	Lilia Talarico	Tom Lategan	Changed to Serial Number 195
IND 195	12/22/98	Lilia Talarico	Tom Lategan	Protocol Amendment: new investigators for the study entitled "The influence of dose and kidney function on bivalirudin pharmacokinetics and pharmacodynamics in patients undergoing Percutaneous coronary artery angioplasty (PTCA); investigators are White; Andrews; Aylward
IND 196	12/23/98	Lilia Talarico	Tom Lategan	Protocol Amendment (New) entitled "An Open Label Study of Bivalirudin for Heparin-Induced Thrombocytopenia (HIT) or Heparin-Induced Thrombocytopenia and Thrombosis Syndrome (HITTS) in Patients Undergoing Percutaneous Coronary Intervention (TMC-98-10)
	1/4/99	Clive Meanwell	Julie DuBeau	Request the batch numbers for the pre-qualification batches
IND 197	1/4/99	Julie DuBeau	John D. Richards	Response to FDA request of January 4, 1999 for batch numbers
	1/11/99	Julieann DuBeau	Tom Lategan	Eric Topol will join the meeting on January 15, 1999
IND 198	1/13/99	Lilia Talarico	Tom Lategan	Protocol Amendment (NEW) entitled "The Effect of Bivalirudin in Combination with Ticlopidine on Laboratory Coagulation Parameters and the Incidence of Clinically Significant Bleeding in Patients Undergoing Percutaneous Intervention and Stenting"
	1/13/99	Tom Lategan	Lilia Talarico	Proposed proprietary name Angiomax is unacceptable
	1/15/99	Julie DuBeau	Tom Wright	Clarify request of January 4 for batch records
	1/15/99	FDA	TMC	Meeting minutes of January 15, 1999; Post-Issuance Not Approvable Letter
	1/22/99	Tom Lategan	Julieann DuBeau	Request that the production (batch) records for the two pre-validation batches be sent to them for review before the batches are released for use in clinical trials.
	1/25/99	Tom Lategan	Julieann DuBeau	Request to contact Dr. Sankoh per Dr. Temples request
	1/26/99	Julieann DuBeau	Tom Lategan	Serial #200 contains the requested batch record
IND 199	1/26/99	Lilia Talarico	Tom Lategan	Changed to Serial Number 200
IND 200	1/26/99	Lilia Talarico	Tom Lategan	1998 Annual Report
IND 201	1/26/99	E. Duffy A. Shaw	John D. Richards	Response to FDA request of January 22, 1999 for the batch record for lot 0931-10-68624
NDA 016	1/26/99	Lilia Talarico	Tom Lategan	Request Teleconference with Statisticians to gain the concurrence of the reviewing statisticians, of the methodology used to impute a placebo event rate, and the resulting argument for non-inferiority of heparin.
IND 202	1/27/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Cortes; Jovane; Geer; Jaume; Rodriguez; Mody; Remington; Brieger; Durech; Ewart; Ekin; Fell; Charles; Nitkin; Zadra; Ikram; Kelleher; Leitch; Newman; Parmar; Rajakumar; Sampson)
IND 203	1/28/99	Lilia Talarico	Tom Lategan	Protocol Amendment entitled "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation Parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA

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Submission Number	Date	To	From	Content
	1/28/99	Julieann DuBeau	Tom Lategan	Communication from MedAdNews concerning the non-approval letter
	2/2/99	Tom Lategan	Julieann DuBeau	Dr. Shaw has completed review of batch record submitted as serial 200 and has passed it to Eric Duffy.
	2/3/99	Lilia Talarico	Neal Kleiman	Request a single patient IND number. Number is 56,484.
	2/4/99	Julie DuBeau	Tom Lategan	HERO-2 and chromogenic assay questions
NDA 017	2/4/99	Lilia Talarico	Tom Lategan	Response to FDA Request for the statistical methods used to establish that Bivalirudin is not inferior to heparin.
	2/5/99	Tom Lategan	Eric P. Duffy	Recommendations / requests regarding batch records
	2/5/99	Julie DuBeau	Tim Lategan	IND Serial numbers out of sequence; next number should be 206
IND 204	2/5/99	Lilia Talarico	Tom Lategan	Changed to Serial Number 205
IND 205	2/5/99	Lilia Talarico	Tom Lategan	Response to FDA request of February 4, 1999 (Teleconference) for information concerning the monitoring of coagulation status during the HERO-2 study
	2/9/99	Tom Lategan	Lilia Talarico	FDA meeting minutes of January 15, 1999
	2/11/99	Tom Lategan	Lilia Talarico	Recommendations/requests regarding protocol TMC 98-10)
NDA 018	2/24/99	Lilia Talarico	Tom Lategan	Appeal to the Nomenclature Committee regarding the use of Angiomax as the trade name for Bivalirudin.
NDA 019	3/3/99	Lilia Talarico	Clive Meanwell	Meeting minutes: January 15, 1999 regarding further activities required to enable approval... (amendment #14)
NDA 020	3/3/99	Lilia Talarico	Clive Meanwell	Response to FDA request for analyses estimating clinical effect of Bivalirudin and heparin compared to an imputed placebo in PTCA and summarize the clinical effects data from Phase II studies in PTCA and unstable angina.
IND 206	3/8/99	Lilia Talarico	Barbara Finn	Protocol Amendment: New Investigators for HERO-2 (Lopez; Alvarez; Amuchastegui; Andres; Arballo; Arrieta; Audeau; Baeff; Barcudi; Bernardo; Birkenheier; Bohorquez; Bonanno; Bono; Botha; Brown; Cagnolatti; Caime; Carroll; Castro; Celsi; Chapidze; Cid; Cinteza; Cisneros; Costamagna; Covelli; Cuello; Datcu; Davies; De Leauw; De Meester; Del Rio; Dumont; Duran; Etchepare; Eusse; Fava; Flores (Juan); Flores (Luis); Friedlander; Fuselli; Gamen; Garcia; Garrahy; Garrido; Gerardo; Giachello; Granados de Arango; Guerrero; Habib; Hasbani; Heredia; Hernandez (Edgar); Hernandez (Ignacio); Hill; Hills; Hrabar; Fernandez; Jeffery; Kindler; Ledesma; Leiva; Liprandi; Longo; Lowenstein; Mamatsashvili; Mann; Marinesco; Martellotto; Marquez; Castellanos; Marzetti; Gambarte; Masino; Medley; Meneghett; Meola; Mezzina; Missault; Morillo; Muntaner; Nelson; Nodar; Nordaby; Olivello; Paganini; Pellegini; Perez; Peroni; Perrino; Pino; Plocek; Queirel; Quijano (Guillermo); Quijano (Alexis); Quinn; Ramos; Restrepo; Rodriguez; Sanchez; Sanchez de Illia; Schneider; Schmitt; Schuster; Scott; Schulte-Herbruggen; Singh; State; Stepanek; Stickland; Suasnabar; Tajer; Tassano; Tatishvili; Tinetti; Toree; Trivi; Tsi
	3/12/99	Tom Lategan	Lilia Talarico	Recommendations and requests regarding protocol entitled "The influence of dose and kidney function on bivalirudin pharmacokinetics and pharmacodynamics in patients undergoing Percutaneous coronary artery angioplasty (PTCA).
IND 207	3/17/99	Lilia Talarico	Tom Lategan	Protocol Amendment; New Investigators for study entitled "The Effect of Bivalirudin in Combination with Ticlopidine on Laboratory Coagulation Parameters and the Incidence of Clinically Significant Bleeding in Patients Undergoing Percutaneous Intervention and Stenting (White; Aylward)
IND 208	3/26/99	Lilia Talarico	Tom Lategan	Response to FDA request of March 12, 1999 regarding protocol "The influence of dose and kidney function on bivalirudin pharmacokinetics and pharmacodynamics in patients undergoing Percutaneous coronary artery angioplasty (PTCA)" submitted as amendment #188 dated December 2, 1998
IND 210	4/2/99	Lilia Talarico	Tom Lategan	Protocol Amendment to revise protocol entitled "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation Parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"

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Submission Number	Date	To	From	Content
IND 209	4/5/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Adrianza; Amarista; Avila; Babes; Bahit; Nogareda; Balestrini; Romera; Caccavo; Cagide; Thierer; Campeanu; Colmenares; Counsell; Criado; Da Costa; Deyanira; Duris; Eibar; Esponera; Gambarte; Girotti; Carbajales; Gomez; Gonzalez; Grancelli; Hedley; Herrera; Hill; Horowitz; Ibarzabal; Isea-perez; Lopez; Kobulia; Magni; Cordoba; Martino; Ulloa; Navarro Nisanci; Owensby; Pacheco; Ponte; Porterie; Rowe; Ryba; Kevorkian; Salazar; Sanchez; Soifer; Tellez; Torres; Tsoi; van Langeveld; Velasquez; Wakley; Fell)
NDA 021	4/22/99	Lilia Talarico	Tom Lategan	Class 1 Resubmission based on the FDA Action Letter of November 18, 1998. Resubmission provides a complete response to the deficiencies noted in the action letter.
IND 211	4/26/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Rodriguez-Ospina; Bastianelli; Boehmer; Craiu; Dvorak; Gordon; Jansky; Kochkarev; Melchior; Melin; Moser; Savage; Tatu-Chitoiu; Uebis; Iseghem; Veze; Zimmermann; Zvejniece)
NDA 022-1	4/26/99	Lilia Talarico	Tom Lategan	Response to FDA telephone request on April 22, 1999 regarding Class 1 Resubmission. The following information is needed to complete the resubmission process. Original Signatures on letter and 356h form; additional information regarding the package label; updated facilities information pertaining to CMC; and an additional copy of volume 2.001, 2.002, 2.003, 2.004 and 2.005.
IND 212	4/28/99	Lilia Talarico	Tom Lategan	Protocol Amendment; New Investigators for study TMC-98-10 (Dauber; Berkowitz; Gilchrist; Dauber; Smith)
	4/29/99	Tom Lategan	Lilia Talarico	Resubmission is considered a complete class 2 response to FDA action letter.
IND 213	4/29/99	Lilia Talarico	Tom Lategan	Protocol Amendment entitled "An Open Label Study of Bivalirudin for Heparin-Induced Thrombocytopenia (HIT) or Heparin-Induced Thrombocytopenia and Thrombosis Syndrome (HITS) in Patients Undergoing Percutaneous Coronary Intervention (TMC-98-10)
IND 214	5/4/99	Lilia Talarico	Tom Lategan	Protocol Amendment to revise protocol "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"
IND 215	5/6/99	Lilia Talarico	Tom Lategan	Protocol Amendment (New) entitled "An Open-Label, Randomized Trial of the Effect of Hirulog (bivalirudin) Following Treatment with Low Molecular Weight Heparin on Laboratory Coagulation Parameters and the Incidence of Adverse Events in Patients Undergoing Percutaneous Coronary Intervention"
NDA 022-2	5/7/99	Julieann DuBeau	Tom Lategan	Follow-up to May 6, 1999 Teleconference regarding the Renal Impairment Study (refer to IND for information)
	4/13/99	Tom Lategan	Lilia Talarico	Comments / recommendations regarding protocol entitled "The Effect of Bivalirudin in Combination with Ticlopidine on Laboratory Coagulation Parameters and the Incidence of Clinically Significant Bleeding in Patients Undergoing Percutaneous Intervention and Stenting"
IND 216	5/17/99	Lilia Talarico	Tom Lategan	Protocol Amendment; New Investigators for study entitled "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation Parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA" (Harrington)
IND 217	5/24/99	Lilia Talarico	Tom Lategan	Protocol Amendment to revise the protocol "An Open-Label Randomized Trial of the Effect of Hirulog (bivalirudin) Following Treatment with Low Molecular Weight Heparin on Laboratory Coagulation Parameters and the Incidence of Adverse Events in Patients Undergoing Percutaneous Coronary Intervention"
IND 218	5/24/99	Lilia Talarico	Tom Lategan	Protocol Amendment to revise the protocol "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"
	5/27/99	Lilia Talarico	Clive Meanwell	Approaches to the assessment of safety and efficacy of bivalirudin as an anticoagulant to be used in patients with unstable angina undergoing PTCA.

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Submission Number	Date	To	From	Content
IND 219	5/28/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Janicke; Young; Tawam; Alejos Mex; Alvarado Ruiz; Arzola; Nevarez; Caceres; Boyarkin; Repin; Boytsov; Dezyugin; Bzhelyanskaya; Fierro; Caglar; Calvillo; Carpio; Claessens; Camara; Carazo; Diaz; de Montoreano; Deger; Demirtas; Dortlemez; Cecena; Ermoshkina; Kalinina; Ferrari; Castillo; Goloshchekin; Galina; Consoreik; Henderson; Garcia; Lopez; Santamaria; Irklienko; Khrakovsky; Alexandr; Ramirez; Herrera; Madrid; Malinin; Sanches; Hernandez; Nefedov; Nikiforova; Ragozina; Ruiz; Ormann; Krahnstover; Ortega; Oto; Carrasco; Acosta; Quintana; Radionov; Shulman; Rajakumar; Corrales; Rifel; Sampo; Hoz; Shvarts; Sirek; Sorokin; Inyushin; Stefanenko; Sumin; Timofeev; Buligin; Timuralp; Turkoglu; Tyrenko; Tytryumov; Arreola; Vasyuk; Sviridov; Vaterlaws; Velarde; Vishnevsky; Teplyakov; Vyorkin; Laptev; Weeks)
IND 220	6/2/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise protocol "The Influence of Dose, Gender and Kidney Function on Bivalirudin Pharmacokinetics and Pharmacodynamics in Patients Undergoing Percutaneous Transluminal Angioplasty"
IND 221	6/2/99	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Investigators to protocol "The Influence of Dose, Gender and Kidney Function on Bivalirudin Pharmacokinetics and Pharmacodynamics in Patients Undergoing Percutaneous Transluminal Angioplasty" (Bittl)
IND 222	6/2/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise protocol "An Open Prospectively Randomized Comparison of Hirulog Versus Heparin in Patients Receiving Aspirin and Thrombolysis (Streptokinase) for the Treatment of Acute Myocardial Infarction: The Hirulog Early Reperfusion/Occlusion (HERO-2) Trial
	6/4/99	Clive Meanwell	Lilia Talarico	Please find the letter concerning bivalirudin.
IND 223	6/16/99	Lilia Talarico	Barbara Finn	Safety Report #TTMC222 occurring in the HERO-2 study.
IND 224	6/15/99	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Investigators for protocol "An Open-Label Randomized Trial of the Effect of Hirulog (bivalirudin) Following Treatment with Low Molecular Weight Heparin on laboratory Coagulation Parameters and the Incidence of Adverse Events in Patients Undergoing Percutaneous Coronary Intervention" (Wallentin; Throvinger; Olsson; Pripp; Sjogren; Berglund; Eriksson; Lindvall)
IND 225	6/16/99	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Investigators for study TMC-98-10 (Rodriguez; Deutsch; Lewis; Mann; Anderson; Ferguson; Gammon; O'Neill)
IND 226	6/21/99	Lilia Talarico	Barbara Finn	Safety Report #TTMC222 occurring in the Cachet Pilot Study; originally reported in error to the HERO-2 study submitted as amendment 223 dated June 16, 1999
	6/21/99	Tom Lategan	Kati Johnson	Proprietary name Angiomax acceptable
NDA 023	6/22/99	Lilia Talarico	John D. Richards	Updated results of chemical testing which demonstrate equivalence of material used in Phase III studies and marketed formulations
IND 227	6/24/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Adgey; Amerena; Barbarash; Tarasov; Belenky; Dascalov; Dohery; Eha; Finnegan; Fotyanov; Tatyana; Golikov; Bykova; Guneri; Karlocai; Ketchker; Tankhilevitch; Lalmond; Lapin; Popov; Lewandowska-Stanek; Liszewsua-Pfejfer; Makhnov; Ironosov; Markov; Vyshlov; Muthusamy; Nechepurenko; Orlikova; Selskov; Panov; Vakhromeeva; Pomogalova; Musurok; Quinonez; Reinhart; Salusbury-Trelawny; Sergeeva; Kim; Shmyzova; Lesnov; Situikova; Khabarov; Strahan; Strekalovsky; Dobrodeev; Szaboki; Teesalu; Timar; Trouerbach; Tumarov; Belehov; Ulusoy; Vahula; Zverv; Lobanov)
IND 228	6/25/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise protocol "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation Parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"
IND 229	6/25/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise protocol "The Effect of Bivalirudin in Combination with Ticlopidine on Laboratory Coagulation Parameters and the Incidence of Clinically Significant Bleeding in Patients Undergoing Percutaneous Intervention and Stenting"
IND 230	6/28/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise the protocol "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation Parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"

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Submission Number	Date	To	From	Content
IND 231	7/1/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC226 occurring in the HERO-2 study submitted as amendment 169
IND 232	7/1/99	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Investigators in study TMC-98-10 (Hassell; Welsh)
IND 233	7/7/99	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Investigators in study TMC-98-10 (Slater)
IND 234	7/14/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise protocol "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation Parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"
	7/20/99	Tom Lategan	Kati Johnson	Comments / information regarding the review of the Clinical and Statistical sections of NDA
IND 235	7/22/99	Lilia Talarico	Tom Lategan	Response to FDA correspondence of June 21, 1999 regarding NDA 20-873 and our proposed proprietary name, Angiomax
NDA 024	7/22/99	Julieann DuBeau	Tom Lategan	Request to formally change name trade name from Hirulog to Angiomax
IND 236	7/27/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Cragg; Sampson; Schmitt; Bett; Nau; Higa; Rajakumar; Turabian; Vermlyen; Celen; Woo)
IND 237	7/28/99	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Investigators for TMC-98-10 (Lincoff; Anderson)
NDA 025	8/5/99	Lilia Talarico	Tom Lategan	Response to FDA request for Information of July 20, 1999 regarding microbiological integrity of the container-closure system; Acute rat study, which was requested by the European Health Authorities statement that the results of this study do not in any way alter the conclusions of our previous studies. The effects are entirely consistent with its pharmacology and known action; Response to FDA letter of July 20 requesting analysis; Safety Update.
IND 238	8/17/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC232 occurring in HERO-2 submitted as amendment #169
IND 239	8/24/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC232 occurring in HERO-2 submitted as amendment #169
IND 240	8/24/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise protocol "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation Parameters and Incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"
IND 241	8/24/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators in HERO-2 (Igartua; Nieves; Aroney)
IND 242	9/1/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC234 occurring in HERO-2 submitted in amendment #169
IND 243	9/8/99	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Investigators in TMC-98-10 (Letcher)
	9/9/99	Julieann DuBeau	Tom Lategan	Package Insert Text
	9/13/99	Victor Raczowski	Clive A. Meanwell	Discussions of the review process
NDA 026	9/15/99	Julieann DuBeau	Tom Lategan	Response to FDA request for Information regarding amendment 023 CMC: updated chemical testing demonstrating equivalence of material used in Phase III studies and marketed formulations; as well as submitting original report for the chromogenic assay for the lot samples (67A04Z, 67A01W; 102052; provide the mannitol content as requested in the May 6 teleconference; provide a comparison of the batch size of lot 0931-10-102052 and the proposed commercial scale; Was lot number 42376 of Bivalirudin as used in TMC 98-09 (renal impairment study) the "to-be-marketed" formulation, which was obtained from a full scale production size batch make by the exact manufacturing procedures and at the same manufacturing site where the "to-be-marketed" formulation is to be made; When is study TMC98-09 expected to be complete, and when will the final study report be submitted.
NDA 027	9/16/99	Julieann DuBeau	Tom Lategan	Response to FDA request for CMC Information: Provide a copy of the batch record for lot 67A01Q, or describe its location in a previous submission; Explain if the lot was diluted due to problems with dissolution; Provide dates for Amendments 21 and 22 as described in the June 22 submission #23.
NDA 028	9/17/99	Julieann DuBeau	Tom Lategan	Response to FDA request of September 14 and 16, 1999 for CMC Information: supplied copy of batch record 67A01Q as requested.

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NDA 029	9/17/99	Julieann DuBeau	Tom Lategan	Response to FDA request for CMC Information: Provide a copy of batch record for 67A02Q, or provide details of its location in the NDA; How can the 9-ASP analog content be reported if it is not part of the actual calculation directions for Method 9310024B (Volume 2.003, Page 189); In the specifications on Page 002 of Volume 2.003 the acceptance criterion is "no other single impurity >1.0%". However, the calculation for Method 9310024B has no provision to report this value. The only other impurity reported is the "major impurities peak occurring at a relative retention time (RRG) of approximately 1.04". How is the "other individual impurity" reported; In the report from the Foundation of Neurologic Diseases in Attachment 2 of June 22 amendment #023, how was the "other individual impurity obtained; Request Dr. Wolfe at the Foundation for Neurologic Diseases to reanalyze the data to include the "Major Impurities Peak" at the RRT 1.04 and "Total Other Impurities". The latter calculation would be for the sum of all non-bivalirudin peaks besides the 9-ASP analog and the "Major Impurity Peak" at RRT
NDA 030	9/17/99	Julieann DuBeau	Tom Lategan	Response to FDA request for CMC Information: Dr. Wolfe's reanalysis of the "Major Impurities Peak" at the RRT 1.04 and "Total Other Impurities". The latter calculation would be for the sum of all non-bivalirudin peaks besides the 9-ASP analog and the "Major Impurity Peak" at RRT 1.04.
IND 244	9/20/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC234 occurring in HERO-2 submitted in amendment #169
NDA 031	9/20/99	Julieann DuBeau	Phyllis Collins	Response to FDA request for CMC Information: Copy of Batch Record 67A02Q as discussed in Amendment #29.
NDA 032	9/21/99	Julieann DuBeau	John D. Richards	Response to FDA request for CMC Information: What data is available to compare lot 67A02Q the lot used in the Bioequivalence testing with the other lots whose assay data is provided in Amendment #23 dated June 22, 1999; How come the peptide concentration in 67A01Q is 0.33 mg/ml when the conc. Is approx. 0.15 mg/ml for the other lots in Amendment #23 dated June 22, 1999. What was the peptide concentration measured by UV275 for lot 67A02Q.
NDA 033	9/24/99	Julieann DuBeau	Phyllis Collins	Response to FDA request of September 22, 1999 for CMC Information: Provide the batch release data (HPLC etc) for the lots 67A04Z, 67A01Q and 67A02Q; Provide actual assay records for the anti-thrombin assays reported in Amendment #23 dated June 22, 1999 or explain why the peptide concentration for lot 67A01Q was 0.33 mg/ml; and why the range of peptide concentrations was so brad for lot 102052; With respect to Question 2 of Amendment #32 dated September 21, 1999, the concentration was 0.5 not 0.15.
NDA 034	9/27/99	Julieann DuBeau	Phyllis Collins	Response to FDA request of September 24, 1999 for CMC Information: Provide the analytical data comparing lot numbers 67A01Q, 67A02Q and 102052. In addition please comment as to whether the HPLC data was generated from the "new HPLC" methods that detect the D-Phe and Asp-9 impurities; Provide the area % of the second largest unknown impurity peak.
IND 245	9/29/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators in HERO-2 (Gamez; Biedermann; Birand; Byrdziak; Saadi; Cooper; Csanady; Daniels; Graaf; Faber; Fitzpatrick; Gossar; Halaczkiwicz; Hiczkiewicz; Horrigan; Alvarado; Jordant; Karidis; Kawka-Urbanek; Klenina; Kozhikina; Koppicz; Krawczyk; Grelecki; Krzeminska-Pakula; Kuc; Kuijer; Belancourt; Lombana; Kignian; Luciard; Calvo; Majcher; Manolis; Novozhenina; Znaenko; Opolski; Oze; Sepulveda; Pieters; Popeye; Prastowski; Ronkowski; Russell; Steinbach; Kindt; Verloove; Ujda; van Nes; Vazquez; Vorochnina; Zelichenok; Vossbeck; Wilkinson; Zawilska; Zemtsovsky; Bondarev; Zinka)
NDA 035	9/29/99			Response to FDA letter dated October 28, 1999: Notice of Intention to Append Application to address deficiencies listed.
	10/8/99	Tom Lategan	Vincent Bille	UCB deficiency letter dated October 1.
	10/8/99	Victor Raczkowski	Clive A. Meanwell	Galley proofs of a paper to be published in Circulation.
	10/8/99	Lilia Talarico	Clive A. Meanwell	Galley proofs of a paper to be published in Circulation.
	10/14/99	John Richards	Nathalie Dubois	Response to FDA request to inspect BioReliance, Scotland

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Submission Number	Date	To	From	Content
	10/15/99	Victor Raczkowski	Clive Meanwell	Information regarding dose-controlled studies performed in Phase II
	10/16/99	Victor Raczkowski	?	This submission was the Kong galley manuscript of the bivalirudin data overview
	10/26/99	Victor Raczkowski	Clive Meanwell	Consider bivalirudin for the additional indication "Bivalirudin is indicated as an anticoagulant in patients undergoing Percutaneous coronary angioplasty for unstable angina presenting within two weeks of myocardial infarction (Braunwald [1989] Class IC-IIIc).
IND 246	10/27/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators in HERO-2 (Chan; Corsini; Dadez; Diehm; Dragulescu; Hubner; Loan; Serrano; Lockert; Lominadzc; Lopex; Messa; Marco; Papazoglou; Paposhvilli; Sinescu; Stritoni; Ruiz; Zavolozhin; Zrazhevsky; Yakovlev)
	10/28/99	Tom Lategan	Victor Raczkowski	Approvable Letter dated October 28, 1999
	10/29/99	Victor Raczkowski	Tom Lategan	Notice to amend application based on October 28 Approvable Letter
NDA 036	11/2/99	Julieann DuBeau	Tom Lategan	Per discussion with Dr. Victor Raczkowski of October 29, 1999 we request a meeting to identify the appropriate pathway for prompt review and approval of the post-MI indication based on the data in the NDA and the submissions of October 15, 21, 25, and 27, 1999.
NDA 037	11/11/99	Julieann DuBeau	Tom Lategan	Response to FDA Approvability Letter of October 28, 1999.
	11/11/99	F. Lochner	John D. Richards	A copy of the CMC sections of NDA Amendment #37
	11/16/99	Julieann DuBeau	Phyllis Collins	Confirm desire to have meeting with Dr. Temple
IND 247	11/22/99	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators to HERO-2 (Bassand; Beck; Bonneau; Buchholz; Avellaneda; Chabanier; Darremont; Cornaert; El-Agez; Evrard; Freydlina; Kalinina; Furber; Galvani; Juergenz; Giles; Luke; Losov; Nikolay; Mycinski; d'Hautefeuille; Pantov; Parisot; Penn; Perchet; Petrov; Poncelin; Fontuch; Dubrava; Riccitelli; Serra; Rondepierre; Roudaut; Simoens; Shaburishvili; Stork; Torres; Vilarem; Weissberg; Wilczek)
	12/2/99	Tom Lategan	Lilia Talarico	Resubmission is a complete class 2 response to FDA action letter dated October 28, 1999
NDA 038	12/15/99	Lilia Talarico	Clive Meanwell	Follow-up to December 13, 1999 teleconference: Summarize outstanding issues, ask FDA to affirm our understanding and reiterate our intention to address the issues in the pre-meeting package and our plans for meeting on January 25.
IND 248	12/17/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise protocol "The Effect of Hirulog in Combination with ReoPro on Laboratory Coagulation parameters and incidence of Clinically Significant Bleeding in Patients Undergoing PTCA"
IND 249	12/20/99	Lilia Talarico	Phyllis Collins	DSMB - 1 st report HERO-2
IND 250	12/20/99	Lilia Talarico	Phyllis Collins	Protocol Amendment to revise protocol "The Influence of Dose, Gender and Kidney Function on Bivalirudin Pharmacokinetics and Pharmacodynamics in Patients Undergoing Percutaneous Transluminal Angioplasty"
NDA 039	12/20/99	Lilia Talarico	Phyllis Collins	HERO-2 DSMB Report: 1 st review
NDA 040	12/20/99	Lilia Talarico	Phyllis Collins	Protocol Amendment: The influence of Dose, Gender and Kidney Function on Bivalirudin Pharmacokinetics and Pharmacodynamics in Patients Undergoing Percutaneous Transluminal Angioplasty. The original protocol was submitted to IND #35,756 as amendment #188 on December 2, 1998 and #22 dated June 2, 1999. This amendment is in response to the FDA October 28, 1999 approvable letter for this NDA in which the FDA requested that the protocol be amended to include patients with more compromised renal impairment.
IND 251	12/22/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC241 occurring in the Cachet study submitted as amendment #169
IND 252	12/22/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC242 occurring in the Cachet study submitted as amendment #169

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IND 253	12/23/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC243 occurring in the HERO-2 study submitted as amendment #169
IND 254	12/30/99	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC245 occurring in the HERO-2 study submitted as amendment #169
NDA 041	1/7/00	Julieann DuBeau	Tom Lategan	January 25, 2000 Briefing Package: Meeting to discuss the approvability of Angiomax for the indication "...as an anticoagulant in patients undergoing Percutaneous coronary angioplasty for unstable angina presenting within two weeks of myocardial infarction" as previously submitted in Amendment #37 dated November 11, 1999.
IND 255	1/17/00	Lilia Talarico	Barbara Finn	Updating transfer of Regulatory Obligations concerning HERO-2
NDA 042	1/19/00	Julieann DuBeau	Tom Lategan	Revised questions for meeting of January 25, 2000
IND 256	1/21/00	Lilia Talarico	Barbara Finn	SAE Line Listing for HERO-2: All adverse events occurring through December 16, 1999
IND 257	1/25/00	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators in HERO-2 (Baldacci; Barbarich; Mihalkova; Carda; Dinnyes; Dostal; Edes; Soltesz; Goede; Jokl; Kermova; Koch; Kviakidis; Mantov; Mlczoch; Mrochek; Adzerikho; Ninova; Oettel; Ogorek; Orfanidis; Piotrowski; Podkhomutnikov; Silina; Pogorelov; Kadochkina; Moreno; Perez; Toral; Wely; Riebartsch; Ronaszeki; Rose; Rozanskiy; Scherbakhin; Sala; Santopinto; Seabra-Gomes; Shalaev; Semuhin; Sinisi; Soroka; Mitkovskaya; Tyurin; Vancik; Vanderheyden; Vardas; Vintila; Zaharouli; Zamolyi)
NDA 043	1/28/00	Julieann DuBeau	Tom Lategan	Response to FDA request of January 27, 2000 for Clinical Information: Provide individual patient information for study C92-304.
IND 258	2/2/00	Lilia Talarico	Phyllis Collins	1999 Annual Report
IND 259	2/8/00	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC248 occurring in HERO-2 submitted as amendment #169
NDA 044	2/14/00	Lilia Talarico	Phyllis Collins	Response to FDA request: supplied copy of Canadian labeling.
NDA 045	2/24/00	Julieann DuBeau	Phyllis Collins	Response to FDA request of February 24, 2000 for Package Labels (tertiary, secondary and vial).
	3/2/00	Tom Lategan	Julieann DuBeau	FDA minutes from February 4, 2000 meeting
NDA 046	3/25/00	Julieann DuBeau	Clive Meanwell	Response to FDA request of February 4, 2000 (meeting) for Clinical Information: submitted assessment of assay sensitivity for Trial C92-304 with respect to open-label heparin; evidence for heparin effectiveness in unstable angina and PTCA; Cachet clinical study report, post-text supplements and clinical study report appendices.
IND 260	3/28/00	Lilia Talarico	Phyllis Collins	DSMB -- 2 nd report HERO-2
IND 261	3/28/00	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Investigators for study "The Influence of Dose and Kidney Function on Bivalirudin Pharmacokinetics and Pharmacodynamics in Patients Undergoing Percutaneous Coronary Artery Angioplasty (PTCA)" (subinvestigators for Dr. White)
	3/28/00	Phyllis Collins	Julieann DuBeau	Three points as a result of FDA meeting regarding Amendment NDA 046 submitted on March 15
NDA 047	3/28/00	Lilia Talarico	Phyllis Collins	Response to FDA request of February 4, 2000 (meeting) for Clinical Information: submitted assessment of assay sensitivity for Trial C92-304 with respect to open-label heparin; evidence for heparin effectiveness in unstable angina and PTCA; Cachet clinical study report, post-text supplements and clinical study report appendices.
IND 262	3/29/00	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Harber; Beinart; O'Sullivan)
NDA 048	3/30/00	Julieann DuBeau	Phyllis Collins	Confirmation from BVL confirming they are ready for FDA inspection.
NDA 049	4/6/00	Julieann DuBeau	John Richards	Updated stability information on final drug product; updated reference letter to DMF10095 from Algroup Wheaton for tubing vials.
	4/6/00	Julieann DuBeau	Phyllis Collins	BVL ready for inspection
	4/7/00	Phyllis Collins	Julieann DuBeau	Status of renal impairment study
NDA 050	4/10/00	Julieann DuBeau	Phyllis Collins	Response to FDA request of April 7, 2000 for the final study report for study "The influence of dose, gender and kidney function on Bivalirudin pharmacokinetics and pharmacodynamics in patients undergoing Percutaneous transluminal angioplasty (TMC 98-09)
	4/13/00	UCB-BioReliance	Liang Zhou	DMF 12797

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IND 263	4/14/00	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Protocol entitled "A comparison of the pharmacodynamic anticoagulant profile of the direct thrombin inhibitor Angiomax (bivalirudin) with heparin after single bolus administration in patients undergoing coronary artery angioplasty and Investigator Information (Ormiston; Webster; Devlin; Simmonds; Abernethy; Elliott; Wilkins)
	4/19/00	Ian Fier	John Hunt	Questions regarding IND #50, final study report for protocol TMC 98-09 entitled "The influence of dose, gender and kidney function on Bivalirudin pharmacokinetics and pharmacodynamics in patients undergoing Percutaneous transluminal angioplasty"
	4/19/00	Ian Fier	John Hunt	Two additional questions regarding study report for protocol TMC 98-09
NDA 051	4/25/00	Julieann DuBeau	Phyllis Collins	Response to FDA's request of April 19, 2000 concerning the study "The influence of dose, gender and kidney function on Bivalirudin pharmacokinetics and pharmacodynamics in patients undergoing Percutaneous transluminal angioplasty (TMC 98-09) as submitted in Amendment #50.
IND 264	4/26/00	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Vidal; Balado; Carrillo; Conci; Grant; Jose; Lockhandwala; Gonzalez; Rodriguez; Adara; Pais; Pardo; Parikh; Patel; Potthoff; Quinn; Risolo; Schwartzman; Sivakadaksham; Thanikachallam; Cortez)
IND 265	5/5/00	Lilia Talarico	Barbara Finn	SAE line listing through March 15, 2000
	5/11/00	Phyllis Collins	Flourence Houn	Approvable Letter
NDA 052	5/12/00	Julieann DuBeau	Phyllis Collins	Response to FDA's letter of May 11, 2000 and our intention to submit an amendment to address the deficiencies.
NDA 053	5/17/00	Julieann DuBeau	Phyllis Collins	Response to FDA's request of May 17, 2000 regarding CMC: submitted the current version of the BioReliance SOP STBT5005 revision 2 entitled Thrombin Inhibition Assay for Bivalirudin drug substance and Bivalirudin containing drug products.
	5/17/00	Phyllis Collins	Julieann DuBeau	Telephone contact to request a teleconference with BioPharmaceutics reviewer.
	5/17/00	Phyllis Collins	Julieann DuBeau	BVL inspection was being done. Art Shaw was asking for current final assay report be faxed to his attention.
NDA 054	5/17/00	Julieann DuBeau	Phyllis Collins	Request Teleconference with BioPharmaceutics to discuss the deficiencies outlined in the Approvable Action Letter of May 11, 2000.
	5/18/00	John Richards	Julieann DuBeau	Correct fax number for Dr. Shaw
	5/19/00	Phyllis Collins	Julieann DuBeau	HERO-2 SAEs and Request for Teleconference with BioPharmaceutics Reviewer
IND 266	5/23/00	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC259 occurring in HERO-2 submitted as amendment #169
IND 267	5/23/99	Julieann DuBeau	John D. Richards	Protocol Amendment; New Protocol entitled "Bivalirudin pharmacokinetics and pharmacodynamics in patients with severe renal impairment"
IND 268	5/24/00	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Achremczyk; Amosova; Arriaga-nava; Barton; Been; Bobak; Boichev; Bonelli; Trujillo; Murillo; Conradie; Moyano; Dubinski; Dykun; Elenkova; Engelbrecht; Filipensky; Francek; Gurasin; Golobrodko; Guryanov; Sivtsova; Herczeg; Tolgyes; Heyndricx; Hominal; Ippoliti; Ivanusa; Jakic; Jovic; Jukic; Kala; Kojoukharov; Kononenko; Kots; Bobylev; Kraiz; Kramer; Kranjcevic; Kurta; Jusnier; Lang; Larregle; Legkonogov; Lloyd; Macian; Maevska; Petukhov; Manak; Polonetskiy; Silva; Marks; Meniconi; Michail; Valery; Mihator; Mikhailova; Beljaev; Milhov; Mogilevsky; Guisareva; Myburgh; Mynhardt; Naidu; Nociar; Oral; Padovan; Parkhomenko; Perchev; Petrov; Pettinati; Plocek; Popovic; Ramos; Ravazzi; Regos; Romic; Rumboldt; Salomon; Samardzic; Santamaria; Sas; Schinke; Sluka; Spies; Supinski; Suskarin; Tantalo; Thakur; Todorov; Urek; Beck; Vassileva; Vico)
IND 269	5/30/00	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC259 occurring in HERO-2 originally submitted as amendment #266
	5/30/00	Phyllis Collins	Julieann DuBeau	Questions regarding fax and submission of May 23, 2000 (Renal Impairment Protocol)

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		John Richards		
IND 270	6/6/00	Julieann DuBeau	Phyllis Collins	Protocol Amendment; New Protocol entitled "Bivalirudin pharmacokinetics and pharmacodynamics in patients with severe renal impairment (TMC-BIV-00-02); Investigator Information (Robson)
IND 271	6/6/00	Julieann DuBeau	Phyllis Collins	Protocol Amendment; New Investigators in TMC-98-10 (Letcher; Berger; McGrew; Wong)
IND 272	6/13/00	Julieann DuBeau	Phyllis Collins	Protocol Amendment; New Protocol entitled "The Effect of Angiomax in Combination with Integrilin Versus Heparin in Combination with Integrilin on Laboratory Coagulation Parameters and Clinical Outcomes in Patients Undergoing Percutaneous Coronary Intervention"; Investigator Information (Kleiman)
	6/13/00	Phyllis Collins	Julieann DuBeau	Question to the IND. Request complete copy of HERO-2 protocol.
IND 273	6/15/00	Julieann DuBeau	Sonja Barton Loar	Response to FDA request of June 13 for amendment protocol "An open prospectively randomized comparison of Hirulog versus heparin in patients receiving aspirin and thrombolysis (streptokinase) for the treatment of acute myocardial infarction: the Hirulog Early Reperfusion/Occlusion (HERO-2) protocol
IND 274	6/23/00	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC267 occurring in HERO-2 originally submitted as amendment #169
IND 275	6/27/00	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators in HERO-2 (Agarwal; Bongosia; Cirko; Desai; Ismail; Yusoff)
IND 276	6/29/00	Lilia Talarico	Phyllis Collins	Protocol Amendment; New Protocol entitled "The Effect of Angiomax in Combination with Tirofiban Versus Heparin in Combination with Tirofiban on Laboratory Coagulation Parameters and Clinical Outcomes in patients Undergoing Percutaneous Coronary Intervention"
IND 277	7/5/00	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC269 occurring in HERO-2 originally submitted as amendment #169
	7/6/00	Phyllis Collins	Lilia Talarico	Comments and recommendations regarding submission #263 dated April 14, 2000 containing protocol TMC-99-06 entitled "A comparison of the pharmacodynamic anticoagulant profile of the direct thrombin inhibitor Angiomax (bivalirudin) with heparin after single bolus administration in patients undergoing coronary angioplasty."
NDA 055	7/14/00	Florence Houn	Sonja Barton Loar	Response to FDAs May 11, 2000 Approvable Action Letter.
	7/20/00	Sonja Loar	Julieann DuBeau	Complete class 2 response to FDA action letter
	7/20/00	Julieann DuBeau	Sonja Loar	As requested, four PC-format diskettes containing the July 14, 2000 proposed Angiomax labeling.
IND 278	7/25/00	Lilia Talarico	Sonja Loar	Protocol Amendment to revise protocol TMC-BIV-00-03, entitled "The Effect of Angiomax in Combination with Integrilin versus Heparin in Combination with Integrilin on Laboratory Coagulation Parameters and Clinical Outcomes in patients Undergoing Percutaneous Coronary Intervention"
IND 279	7/26/00	Lilia Talarico	Sonja Loar	Response to FDA request of July 6, 2000 regarding clarification on protocol TMC-99-06 entitled "A comparison of the pharmacodynamic anticoagulant profile of the direct thrombin inhibitor Angiomax (bivalirudin) with heparin after single bolus administration in patients undergoing coronary angioplasty"
IND 280	7/28/00	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Adamus; Carrageta; Percira; Chan; Zotiys; Gil; Gordienko; Laurenchenco; Habscheid; Kamenik; Keung; Khzamov; Mostovoy; Reissmann; Rinke; Ruano; Saifutdinov; Timashev; Simon; Sojka; Antolik; Sullivan; Volkova)
	7/31/00	Sonja Loar	Julieann DuBeau	Internal resubmission review meeting will be held 8/21/00.
	7/31/00	Julieann DuBeau	Sonja Loar	Press release which is referenced in our Protocol TMC-BIV-00-03 (serial #272).
	8/2/00	TMC	Jeffrey Weber	User Fee
IND 281	8/4/00	Lilia Talarico	Barbara Finn	Safety Report #TTMC270 occurring in the HERO-2 trial - acute renal failure - initial report.
IND 282	8/10/00	Lilia Talarico	Sonja Loar	Protocol amendment to revise protocol TMC-BIV-00-03, entitled "The effect of Angiomax in combination with Integrilin versus heparin in combination with Integrilin on laboratory coagulation parameters and clinical outcomes in patients undergoing Percutaneous coronary intervention"

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Submission Number	Date	To	From	Content
	8/15/00	Julieann DuBeau	Sonja Loar	Desire to change dosing table to reflect a constant concentration infusion bag.
IND 283	8/18/00	Lilia Talarico	Sonja Loar	Response to FDA request of August 15 (teleconference) regarding predicted hemorrhage/bleeding rates for patients randomized to the Integrilin/heparin arm of study TMC-BIV-00-03; New Investigators for protocol #TMC-BIV-00-04 entitled "The Effect of Angiomax in Combination with Tirofiban versus Heparin in Combination with Tirofiban on Laboratory Coagulation parameters and Clinical Outcomes in Patients Undergoing Coronary Intervention.
	8/21/00	Julieann DuBeau	John Richards	Slides from the ESPRIT trial presented at the ACC meeting.
	8/24/00	Julieann DuBeau	Phyllis Collins	Request feedback on internal meeting held on 8/21/00.
IND 284	8/24/00	Lilia Talarico	Phyllis Collins	SAE Line listing and tables for HERO-2 from March 14 through June 15, 2000
IND 285	8/30/00	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Apffel; Gaul; Gessek; Grishina; Pluta; Pulkowski; Staroverov; Zhurova; Tittbach)
IND 286	9/14/00	Lilia Talarico	Phyllis Collins	DSMB – 3 rd report for HERO-2; New Investigators for study TMC-98-10 (Melnyk; Mahaffey)
IND 287	9/18/00	Lilia Talarico	Barbara Finn	Follow-up Safety Report #TTMC273 occurring in HERO-2 originally submitted as amendment #169
IND 288	9/29/00	Lilia Talarico	Barbara Finn	Protocol Amendment; New Investigators for HERO-2 (Bhuvanewaran; Habaluyas; Parikh; Soon)
	10/2/00	Phyllis Collins	Lilia Talarico	Recommendation regarding submission #272 dated June 13, 2000 containing a new protocol entitled "The Effect of Angiomax in Combination with Integrilin versus Heparin in Combination with Integrilin on Laboratory Coagulation parameters and Clinical Outcomes in patients Undergoing Percutaneous Coronary Intervention.
	10/6/00	Sonja Loar	Julieann DuBeau	Discuss revised Dosage and Administration section; as well as review status.
NDA 056	10/9/00	Lilia Talarico	Sonja Loar	Labeling Revision: Dosage & Administration
IND 289	10/17/00	Lilia Talarico	Lisa-Sue Wood	SAE Line Listing and tables for HERO-2 from June 15 through September 15, 2000
IND 290	10/18/00	Lilia Talarico	Lisa-Sue Wood	Protocol Amendment to revise protocol TMC-BIV-00-03 entitled "The Effect of Angiomax in Combination with Integrilin versus Heparin in Combination with Integrilin on Laboratory coagulation Parameters and Clinical Outcomes in Patients Undergoing Percutaneous Coronary Intervention (third); Investigator Information for study TMC-98-10 (Berger)
	10/18/00	Julieann DuBeau	Sonja Loar	Conference call for Monday, October 23 regarding BioReliance SOP SPBT5005R05, Thrombin Inhibition Assay
	10/18/00	Julieann DuBeau	Sonja Loar	Confirmation regarding participation in Monday's meeting.
	10/19/00	Nadine Ritter	Tom Wright	Request BioReliance participation in Monday's conference call.
	10/20/00	Julieann DuBeau	Lisa-Sue Wood	BioReliance SOP SPBT5005 (all versions)
	10/27/00	Nathalie Dubois	Liang Zhou	FDA letter to UCB-Bioproducts S.A. regarding DMF 12797. FDA requests additional information and UCB response.
	10/30/00	Julieann DuBeau	Sonja Loar	Faxed draft revised BioReliance Thrombin Inhibition Assay. (need to find original fax and attachments)
	10/31/00	Julieann DuBeau	Sonja Loar	Biopharm review is done. CMC and clinical are to have final reviews by end of week. Labeling negotiations possibly next week.
IND 291	11/1/00	Lilia Talarico	Barbara Finn	Protocol Amendment – New Investigators in the HERO-2 study (Panchavinnin, Adaro, Adrianza, Almeida, Amarista, Calvo, Carpio, Castillo, Cortex, De Costa, Diaz, Esponera, Gomex, Lombana, Lopez, Medina, Nava, Pacheco, Perez, Rodriguez, Sanchez, Santamaria, Sepulveda, Torres)
	11/2/00	Julieann DuBeau	Sonja Loar	Everything OK with Biopharm review. She hasn't received notification that the facilities are ok. Angiomax still has to be cleared by OPDRA. Looking at labeling the week after next. Hoping for final approval by end of the year.
IND 292	11/6/00	Lilia Talarico	Sonja Loar	Protocol Amendment: New Protocol TMC-BIV-00-01 Replace; Investigator Information (Lincoff) Packaging Information: Vial label, carton label, PCS Information
	11/7/00	Julieann DuBeau	Sonja Loar	Dr. Shaw has one last question regarding BioReliance Thrombin Inhibition Assay SOP SPBT5005
NDA 057	11/9/00	Lilia Talarico	Sonja Loar	Final approved BioReliance SOP SPBT5005.R06 entitled "Thrombin Inhibition Assay for Bivalirudin Drug Substance and Bivalirudin-Containing Drug Products"

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Submission Number	Date	To	From	Content
	11/9/00	Julieann DuBeau	Sonja Loar	Julie requests a different electronic copy of the Dosage and Administration Table 5. She has still not received official notice of the facilities acceptance. The package can go to Victor without it. All reviews are to be finalized by Monday, November 13. Labeling changes will be drafted before Nov 20. Projected time to go to Victor is November 29.
	11/17/00	Julieann DuBeau	Sonja Loar	Request for Teleconference regarding proprietary name: Angiomax
	11/21/00	Julieann DuBeau	Sonja Loar	Revised request for teleconference regarding proprietary name: Angiomax
IND 293	11/22/00	Lilia Talarico	Lisa Travis	IND Safety Report #TTMC269 – Follow-up to event occurring in HERO-2 originally submitted as amendment #169. Change in contact for Quintiles to Lisa Travis
	11/28/00	Julieann DuBeau	Sonja Loar	Telefax of attendees for teleconference regarding proprietary name Angiomax
	11/28/00	Sonja Loar	Julieann DuBeau	Divisional comments regarding proposed Angiomax Labeling.
IND 294	11/30/00	Lilia Talarico	Lisa Wood	Protocol Amendment: New Investigators for study TMC-BIV-00-01 REPLACE (Deibel, Arora, Bhoopalam, Clark, Imburgia, Le, Moor, Sanz, Sarembock, Giles)
	11/30/00	Sonja Loar	Julieann DuBeau	Phase IV Commitment
IND 295	12/1/00	Lilia Talarico	Lisa Travis	Protocol Amendment: New/Revised Investigators for HERO2 (Bohorquez; Carrillo; Corrales; Garcia; Hernandez; Mex; Nevarez; Restrepo; Silva)
NDA 058	12/1/00	Lilia Talarico	Sonja Loar	Draft Labeling dated December 1 in response to FDA letter of November 28
	12/6/00	Nathalie Dubois	Liang Zhou	4 Additional questions from FDA
	12/8/00	Julieann DuBeau	Sonja Loar	Fax regarding references in labeling.
	12/8/00	Julieann DuBeau	Sonja Loar	e-mail regarding labeling.
	12/8/00	Julieann DuBeau	Sonja Loar	Hardcopy fax of D&A section.
	12/8/00	Julieann DuBeau	Sonja Loar	Fax of referenced pages (labeling)
	12/11/00	Sonja Loar	Julieann DuBeau	Agreed labeling text between the Division and TMC.
	12/ /00	Sonja Loar	Lilia Talarico	Post-approval commitment
IND 296	12/12/00	Lilia Talarico	Lisa Travis	IND Safety report TTMC273 (HERO-2)
IND 297	12/13/00	Lilia Talarico	Lisa Travis	IND Safety report TTMC270 (HERO-2)
	12/15/00	Sonja Loar	Julie DuBeau	APPROVAL of Angiomax
	12/20/00			FDA's posting on their homepage: Angiomax approval
S001	12/20/00	Lilia Talarico	John Richards	18 month expiry data
IND 298	12/22/00	Lilia Talarico	Lisa Wood	REPLACE investigator information
	12/22/00	Dept H&HS	Lisa Wood	Drug listing forms 2657 & 2658 (copies can be found in file room Drug Registration and Listing)
IND 299	12/29/00	Lilia Talarico	Lisa Travis	HERO-2 investigators

Appendix K Calculation of extension

