

Synthesis of $^{13}\text{C}_3$, $^{15}\text{N}_4$ -labeled Factor Xa Inhibitor

William J. Guilford*, Jerry Dallas, and Damian Arnaiz

Medicinal Chemistry, Berlex Biosciences, 15049 San Pablo Avenue,
Richmond California 94804

Abstract: The synthesis of an isotopically-labeled, diphenoxypyridine factor Xa inhibitor, 3-[[6-[3-(4,5-dihydro-1-(^{13}C)-methyl-1H-(4- ^{13}C , $^{15}\text{N}_2$ -imidazol-2-yl)phenoxy)-3,5-difluoro-4-(4-morpholinyl)-2-pyridinyl]oxy]-4-hydroxybenzene- ^{13}C -carbox- ^{15}N -imid- ^{15}N -amide, is reported for use in factor Xa binding studies using REDOR NMR. N-benzyl protected tetralabeled N-methylethylene diamine was an intermediate in the preparation of 3-[4,5-1-(^{13}C)-methyl-1H-(4- ^{13}C , $^{15}\text{N}_2$ -imidazol-2-yl)]phenol.

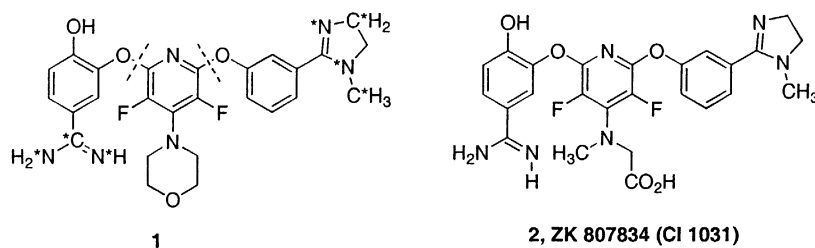
Key Words: Factor Xa inhibitor, ZK 807834 (CI 1031), mass label, tetralabeled N-methyl ethylenediamine

Introduction

There remains an unmet clinical need for a safe, effective, and fast-acting antithrombotic. Factor Xa (fXa), a trypsin-like serine protease that plays a key role in the blood coagulation, is an ideal target for an anticoagulant since it occupies the strategic juncture of the two arms of the coagulation cascade, the intrinsic (surface activated) and the extrinsic (vessel injury-tissue factor) pathway¹. Efforts to identify potent and selective fXa inhibitors have been the subject of recent reviews^{2,3}. As part of our effort to describe the interactions between fXa and our pyridine class of inhibitors, we have employed rotational-echo double resonance, REDOR, NMR

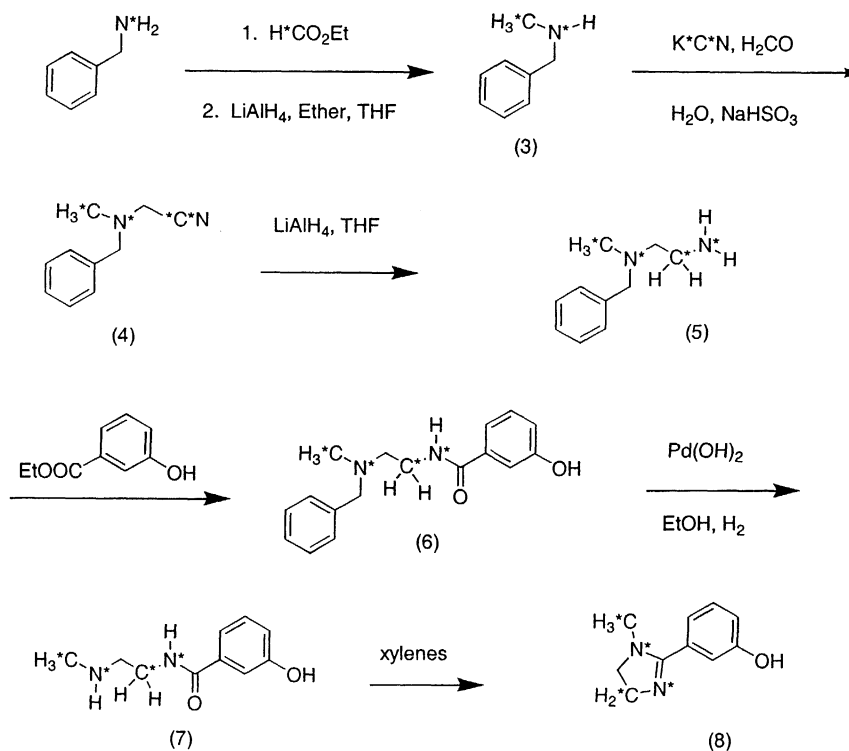
analysis^{4,5} to characterize the conformation of **1** bound to fXa. Compound **1** is an analog of our clinical candidate ZK 807834 (CI 1031), **2**⁶, which bears the two phenoxy groups which interact with fXa, as determined from earlier X-ray⁷ and REDOR NMR studies⁵. We report here the synthesis of ¹³C₃, ¹⁵N₄-labeled analogue (**1**) needed for the REDOR NMR study.

The interatomic distance between ¹³C labeled carbons on the imidazole and amidine groups and natural abundance ¹⁹F atoms on the pyridine ring is interpreted from dipolar couplings measured in a REDOR NMR experiment⁴. The interatomic distances are used to define the overall bound conformation of the inhibitor-fXa complex using molecular modeling⁵. Both the methyl and the methylene carbons on the imidazole moiety were labeled to provide two measured distances for the imidazole moiety. All nitrogen atoms on the amidine and imidazole groups were labeled with ¹⁵N to facilitate double REDOR NMR experiments which select only those ¹³C nuclei bound to ¹⁵N, thus eliminating the overwhelming background natural abundance ¹³C resonances from fXa.



Chemistry

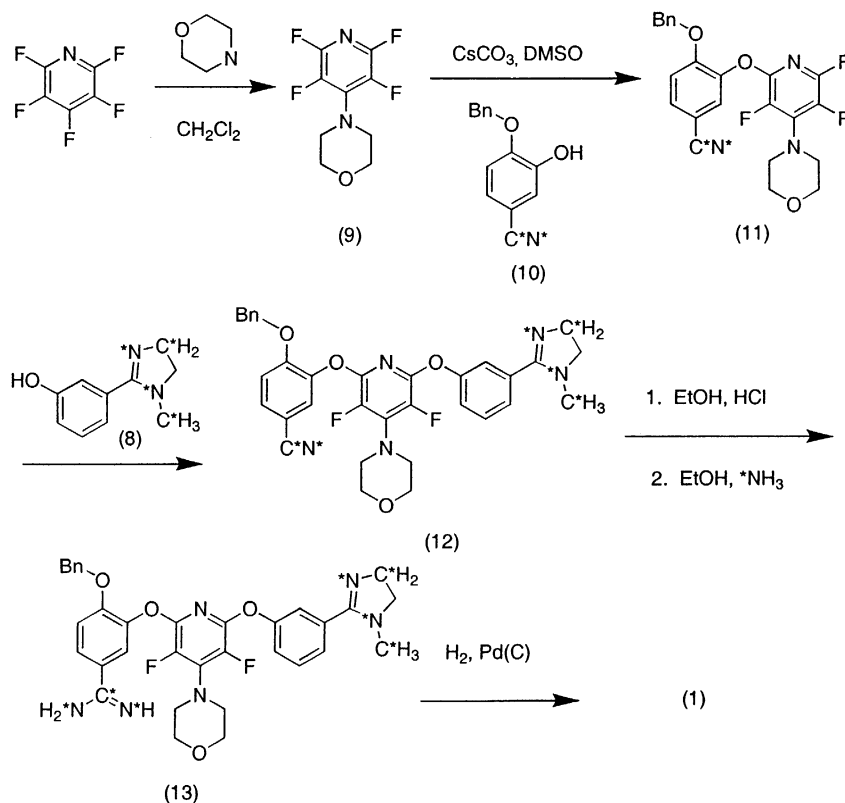
The synthesis of **1** was based on the published synthesis of ZK 807834 (**2**, CI 1031) and is shown in Schemes 1 and 2⁶. The two strategic bond connections are between a suitably substituted phenol and the fluorinated pyridine. The preparation of tetralabelled-phenol **8** requires assembling a five carbon unit with four isotopically-enriched atoms. The synthesis, Scheme 1, was designed to incorporate the labeled atoms from



Scheme 1: Synthesis of mass labeled **8**.

small, commercially available starting materials in a way which minimized losses due to compound volatility or water solubility. Acylation of benzyl(^{15}N)amine with ethyl (^{13}C)formate followed by reduction with lithium aluminum hydride in a mixture of THF and ether gave N-benzyl (^{13}C)methyl(^{15}N)amine (**3**). The Knoevenagel-Bucherer modification of the Strecker reaction using potassium (^{13}C , ^{15}N)cyanide gave the desired tetrasubstituted compound **4**. Reduction of the nitrile using lithium aluminum hydride in THF gave the primary amine, **5**, in near quantitative yield. Acylation of **5** with ethyl hydroxybenzoate by heating the reagents neat in a 120°C oil bath gave **6**. The N-benzyl protecting group was removed by hydrogenation over palladium hydroxide. Heating a xylene solution at reflux with water removal with the aid of a Dean-Stark trap resulted in dehydration of **7** to yield 3-[4,5-dihydro-1-(^{13}C -methyl)-1H-(4- ^{13}C , $^{15}\text{N}_2$ -imidazol-2-yl)]phenol (**8**).

The substitution pattern on the pyridine can be easily established through the order of addition³ (Scheme 2). Treatment of pentafluoropyridine with morpholine in the presence of triethylamine gave the 4-substituted pyridine **9**. Phenol **10** was prepared by alkylation of the commercially prepared 3,4-dihydroxybenzo(¹³C, ¹⁵N)nitrile with benzyl chloride⁸⁹. Coupling of **9** and **10** in DMF using cesium carbonate as a base gave **11**. Addition of phenol **8** to pyridine **11** using cesium carbonate in DMSO and slightly higher temperatures gives diphenoxypyridine **12**. Treatment of **12** with hydrogen chloride in ethanol gave the desired imidate, as well as debenzoylation of about one-third of the material. The mixture of benzylated and debenzylated material was treated (¹⁵N)ammonia in a sealed tube to give a mixture of compounds **1** and **13**. Complete debenzoylation was



Scheme 2. Synthesis of mass labelled **1**.

accomplished with hydrogenation over a Palladium catalyst to yield **1**. Mass spectral analysis of **1** predicted an enrichment of 99% at each of the seven positions. The structure of the **1** and the location of the labeled atoms was confirmed by NMR analysis (see experimental for details).

Conclusion

Compound **1** has been prepared in good overall yield and purity using a modification of the published unlabeled procedure³. In route to **1**, [(^{13}C -methyl)(phenylmethyl)(^{15}N -amino)](2- ^{13}C)ethyl(^{15}N)amine (**5**) was prepared from small, commercially-available precursors in excellent yield. The availability of hepta-labelled **1** will enabled us to proceed with the REDOR NMR. Results of these studies will be reported at a later date.

Experiment Methods

^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a Varian Gemini 400 using TMS as a standard. ^{13}C Chemical shifts are reported only for the labeled carbons. Mass spectral analysis were obtained on a Finnigan LC-Q. Labeled syntheses were carried out initially using unlabeled reagents and gave the desired products in similar purities and yields. Labeled reagents were obtained from Isotec, Inc. (Miamisburg, OH): benzyl(^{15}N)amine (99at. %), 3,4-dihydroxybenzo(^{13}C , ^{15}N)nitrile (99at %) and (^{15}N)ammonia (99at %) and Aldrich: ethyl (^{13}C)formate (99 at. %) and potassium (^{13}C , ^{15}N)cyanide (99 at. %). Analytical HPLC was obtained on a Rainin HPLC using a vydac 218TP54 column with a 1ml/min flow rate, $\lambda = 254$ nm, A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile. Preparative HPLC was obtained on a Rainin HPLC using dynamax R00083241C 41.4 mm ID column, A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile.

N-(^{13}C -methyl)-benzenemethan-(^{15}N)amine (3) Benzyl(^{15}N)amine (2g, 19 mmol) and ethyl (^{13}C)formate (2g, 27 mmol) were stirred and heated in a 70°C oil bath for 16 h. Excess reagent was removed under vacuum and the

resulting white solid, **26**, was isolated: ^1H NMR (CDCl_3) 8.25 (dd, 1H, $J = 200\text{Hz}$), 7.3 (m, 5H), 5.82 (m, 1H, $J = 90\text{Hz}$), 4.48 (t, 2H); ^{13}C NMR (CDCl_3) 165.8, 161.92.

A slurry of lithium aluminum hydride (1.1 g, 30 mmol) in ether (60 mL) was stirred as a solution of (phenylmethyl)(^{13}C , ^{15}N)formamide (2.6 g, 19 mmol) was added in small portions. The reaction was heated at reflux for 6h and allowed to cool. Excess reagent was quenched by the addition of water (1.1 mL), 1N aqueous sodium hydroxide solution (1.1 mL), and water (3.3 mL). The resulting solid was removed by filtration. Aluminum salts were extracted with a mixture of THF and ether. The combined filtrates were concentrated to yield 2.3g of an oil: ^1H NMR (CDCl_3) 7.25 (m, 5H), 3.73 (m, 3H), 2.45 (d, 3H, $J = 200\text{ Hz}$); ^{13}C NMR (CDCl_3) 37.2.

[(^{13}C -methyl)(phenylmethyl)(^{15}N -amino)](2- ^{13}C , ^{15}N)acetonitrile (**4**) A solution of sodium bisulfate (2.6 g, 25 mmol) in water was stirred as formaldehyde (2.3 mL, 28 mmol, 37 wt %) and **3** (2.3g, 19 mmol) were added. The reaction was stirred for 1h, before the addition of potassium (^{13}C , ^{15}N)cyanide (1.7g, 25 mmol). The reaction was placed in a 70°C oil bath and warmed for 2h. The reaction was allowed to cool, diluted with water and extracted with ethyl acetate (3x). The combined organic extracts were washed with water and a brine solution, dried (Na_2SO_4), and concentrated to give 3 g of a light colored oil: ^1H NMR (CDCl_3) 7.3 (m, 5H), 3.63 (s, 2H), 3.44 (s, 2H), 2.45 (d, 3H, $J = 200\text{ Hz}$); ^{13}C NMR (CDCl_3) 117, 43.3; LCMS (ESI) $m/z = \text{Found } 164, \text{C}_8^{13}\text{C}_2\text{H}_{12}^{15}\text{N}_2 (M^+)$ requires 164.

[(^{13}C -methyl)(phenylmethyl)(^{15}N -amino)](2- ^{13}C)ethyl(^{15}N)amine (**5**) A slurry of lithium aluminum hydride (1.5g, 40 mmol) in THF was stirred as a solution of substituted acetonitrile (**4**, 3 g, 19 mmol) was added in small portions. Reaction was heated at reflux for 16 h. The reaction was allowed to cool before the addition of water (1.5 mL), 1N aqueous sodium hydroxide solution (1.5 mL), and water (4.5 mL). The solid was isolated by filtration

and extracted with a mixture of ether and THF. The combined filtrates were concentrated to give 2.9 g: ^1H NMR (CDCl_3) 7.3 (m, 5H), 3.58 (s, 2H), 2.82 (s, 2H, $J = 200\text{Hz}$), 2.5 (m, 2H), 2.28 (d, 3H, $J = 90\text{ Hz}$); ^{13}C NMR (CDCl_3) 43.3, 41.3; LCMS (ESI) $m/z = \text{Found } 168$, $\text{C}_8^{13}\text{C}_2\text{H}_{16}^{15}\text{N}_2$ (M^+) requires 168.

3-Hydroxy-N-[2-[(^{13}C -methyl)(phenylmethyl) (^{15}N -amino)](1- ^{13}C -ethyl)(^{15}N)benzamide (6) A mixture of substituted ethanediamine (5, 2.9 g, 18 mmol) and ethyl hydroxybenzoate (3.2g, 19 mmol) was heated in an 120°C oil bath for 24 h. The reaction was allowed to cool. Purification by column chromatography using a 1 to 5% methanol in ethyl acetate gradient yielded 2.8 g (54%) of the desired product: ^1H NMR (CDCl_3) 7.62 (s, 1H), 7.3 (m, 9H), 6.83 (m, 1H), 3.61 (d, 2H, $J = 200\text{Hz}$), 3.58 (s, 2H), 2.64 (m, 2H), 2.32 (d, 3H, $J = 200\text{ Hz}$); ^{13}C NMR (CDCl_3) 42.8, 38.3; LCMS (ESI) $m/z = \text{Found } 288$, $\text{C}_{15}^{13}\text{C}_2\text{H}_{20}^{15}\text{N}_2\text{O}_2$ (M^+) requires 288.

3-Hydroxy-N-[2-(N- ^{13}C -methyl - ^{15}N -amino)(1- ^{13}C -ethyl)(^{15}N)benzamide (7) A solution of substituted N-benzylamine (6, 2.8g, 9.8 mmol) in ethanol (300 mL) was deoxygenated with nitrogen before the addition of palladium hydroxide (0.56g). The reaction mixture was placed on a Parr Hydrogenator under 42 psi of Hydrogen for 16 h. Catalyst was removed by filtration through celite. Filtrate was concentrated under reduced pressure to give 1.8 g of an oil: ^1H NMR (CDCl_3) 8.2 (d, 1H, $J = 90\text{ Hz}$), 7.2 (m, 3H), 6.82 (m, 1H), 3.32 (m, 2H, $J = 200\text{Hz}$), 2.57 (m, 2H), 2.28 (d, 3H, $J = 200\text{ Hz}$); ^{13}C NMR (CDCl_3) 41.3, 37.8; LCMS (ESI) $m/z = \text{Found } 198$, $\text{C}_8^{13}\text{C}_2\text{H}_{14}^{15}\text{N}_2\text{O}_2$ (M^+) requires 198.

3-[4,5-dihydro-1-(^{13}C -methyl)-1H-(4- ^{13}C , $^{15}\text{N}_2$ -imidazol-2-yl)]phenol (8) A slurry of amine 7 (1.8 g, 9.3 mmol) in xylenes (150 mL) was heated at reflux in a flask equipped with a Dean-Stark trap for 8 h. The reaction was allowed to cool. Solvent was removed under reduced pressure. Residue was recrystallized from hot 2-propanol to give 1.2 g of an off-white solid: ^1H NMR (CDCl_3) 7.2 (m, 1H), 6.82 (m, 2H), 6.78 (m, 1H), 3.62 (dt, 2H, $J =$

200Hz), 3.27 (m, 2H), 2.65 (d, 3H, $J = 200$ Hz); ^{13}C NMR (CDCl_3) 52.8, 36.3; LCMS (ESI) $m/z = \text{Found } 180$, $\text{C}_8^{13}\text{C}_2\text{H}_{12}^{15}\text{N}_2\text{O}$ (M^+) requires 180.

4-morpholinyl-2,3,5,6-tetrafluoropyridine (9) A solution of morpholine (32.8 g, 376 mmol) in dichloromethane (1L) and triethylamine (125 mL, 897 mmol) was cooled to -7°C and stirred as pentafluoropyridine (62g, 370 mmol) was added dropwise over 30 min. The solution was allowed to warm to ambient temperature overnight. The reaction was poured into a 10% aqueous acetic acid solution. The organic layer was dried and concentrated to afford 87 g of **9** as a white solid: ^1H NMR ($\text{DMSO}-d_6$) 3.7 (t, 4H), 3.40 (m, 4H).

6-(phenylmethoxy)-3- ^{13}C , ^{15}N -nitrilephenol (10) Alkylation of 3,4-dihydroxybenzo(^{13}C , ^{15}N)nitrile with benzyl chloride gives 1.3 g (81%) of **10**: HPLC (20-70% B over 30 min) 16.5 min (88%), ^1H NMR (DMSO) 7.4 (m, 6H), 7.20 (m, 1H), 7.16 (m, 1H), 5.20 (s, 2H).

3-[[6-[3-(4,5-dihydro-1-(^{13}C)-methyl-1H-(4- ^{13}C , $^{15}\text{N}_2$ -imidazol-2-yl)phenoxy)-3,5-difluoro-4-(4-morpholinyl)-2-pyridinyl]oxy]-4-(phenylmethoxy)-benzo- ^{13}C , ^{15}N -nitrile (11) A solution of **9** (1.35 g, 5.72 mmol) and **10** (1.30 g, 5.72 mmol) in DMSO (28 mL) was treated with cesium carbonate (4.90 g, 15.04 mmol). The reaction was warmed to 40°C and stirred overnight. The reaction mixture was cooled to room temperature and poured into a 1% aqueous potassium carbonate solution (300 mL). The aqueous layer was extracted with dichloromethane (3 x 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford a white solid. Purification by flash chromatography using a gradient of ethyl acetate in hexane afforded 2.14 g of **3** as a white solid: HPLC (20-70%B over 30 min) 23.38 min (93%).

A stirred solution of **11** (2.14 g, 4.83 mmol) and **8** (1.11 g, 6.19 mmol) in DMSO (35 mL) was treated with cesium carbonate (4.60 g, 14.12 mmol)

and heated at 60°C overnight. The reaction was cooled to room temperature, poured into a 1% aqueous potassium carbonate solution (300 mL), and extracted with dichloromethane (3 x 200 mL). The aqueous layer was concentrated and the resulting residue was washed with dichloromethane. The combined organic layers were washed with 1 % aqueous potassium carbonate (3 x 100 mL), dried (sodium sulfate) and concentrated to afford a dark oil. Purification by flash chromatography (silica gel, hexane-ethyl acetate) gave 2.49 g (89%) of **12** as a yellow oil: HPLC (20-70%B over 30 min): 18.54 min; ^1H NMR (DMSO- d_6) 7.60 (m, 2H), 7.20 (m, 8H), 6.95 (m, 2H), 5.05 (s, 2H), 3.65 (m, 6H), 3.25 (m, 6H), 2.75 (s, 3H).

3-[[6-[3-(4,5-dihydro-1-(^{13}C)-methyl-1H-(4- ^{13}C , $^{15}\text{N}_2$ -imidazol-2-yl)phenoxy)-3,5-difluoro-4-(4-morpholinyl)-2-pyridinyl]oxy]-4-

hydroxybenzene- ^{13}C -carbox- ^{15}N -imid- ^{15}N -amide (1) A solution of **12** (2.49 g, 4.13 mmol) and methanol (50 mL) was cooled to -30 °C. Hydrogen chloride was added slowly keeping the temperature below 0 °C. After the solution was saturated, the reaction was warmed to 0 °C, sealed, and placed in the refrigerator overnight. The reaction was concentrated *in vacuo*. The residue was dissolved in ethanol (50 mL), concentrated under reduced pressure, and dried under high vacuum for 1 h. The residue was dissolved in methanol (75 mL), placed in a sealed tube, cooled to -30°C, and treated with (^{15}N)Ammonia (710 mg, 39.40 mmol). The tube was sealed and warmed to room temperature. The tube was then placed in an oil bath at 40°C overnight. The reaction was cooled and concentrated. Methanol (200 mL) was added to dissolve the residue: HPLC (20-70%B over 30 min) 9.44 min (38%) for **1**, 13.58 min (57%) for **13**. The methanolic solution of **1** and **13** was treated with catalyst, 10% Pd/C NE/W degussa type E101 (310 mg) and placed on the hydrogenation apparatus (30 psi). After 2h, the reaction mixture was filtered through celite and concentrated to an oil (MR 1.55 g). Purification by preparative C18 HPLC afforded 0.91 g of **1** as a white solid after lyophilization: HPLC (20-70%B over 30 min) 9.31 min (97.5%); ^1H

NMR (DMSO-d₆) 11.15 (m, 1H), 10.25 (d, 1H), 8.9 (m, 3H), 7.57 (m, 2H), 7.49 (t, 1H), 7.34 (m, 3H), 7.01 (d, 1H), 4.05 (m, 3H), 3.75 (m, 6H), 3.42 (m, 3H), 2.95 (dd; $J = 141.6$ Hz and $J = 2$ Hz, 3H); ¹³C NMR (DMSO-d₆) 163.6 (t, $J_{\text{CN}} = 19.01$ Hz), 42.6 (d, $J_{\text{CN}} = 5.43$ Hz), 33.9 (d, $J_{\text{CN}} = 10.66$ Hz). LCMS (ESI) 532 (M⁺) C₂₃ ¹³C₃H₂₆F₂N₂¹⁵N₄O₄ requires 532; Anal. (C₂₆H₂₆F₂N₆O₄·2HCl·0.1CF₃CO₂H·2.25H₂O) C, H, N, F.

Acknowledgements: We wish to thank Karen May and Drs. Ken Shaw, Gary Phillips and John Bauman for helpful suggestions and discussions.

References and Notes:

- ¹ Davie, E., Fujikawa, K., Kisiel, W. *Biochem.* **30**, 10363-10370 (1990).
- ² Ewing, W.R., Pauls, H.W., Spada, A.P. *Drugs of the Future* **24**, 771-787 (1999).
- ³ Fevig, J.M., Wexler, R.R. *Annual Reports in Medicinal Chemistry* **34**, 81-100 (1999).
- ⁴ Beusen D. D., McDowell L. M., Slomczynska U., and Schaefer, J. *J. Med. Chem.* **38**: 2742-2747 (1995).
- ⁵ McDowell L. M., McCarrick M. A., Studelska, D. R., Guilford W. J., Arnaiz D., Dallas J. L., Light D. R., Whitlow M., and Schaefer, J. *J. Med. Chem.* **42**: 3910-3918 (1999).
- ⁶ Phillips G. B., Buckman B. O., Davey D. D., Eagan K. A., Guilford W. J., Hinchman J., Ho E., Koovakkat S., Ng H. P., Post J. M., Shaw K. J., Smith D., Subramanyam B., Sullivan M.E., Trinh L., Vergona R., Walters J., White K., Whitlow M., Wu S., Xu W., and Morrissey, M. M. *J. Med. Chem.* **41**: 3557-3562 (1998).
- ⁷ Whitlow M., Arnaiz D., Buckman B. O., Davey D. D., Griedel B., Guilford W. J., Koovakkat S., Liang A., Mohan R., Phillips G. B., Seto M., Shaw K. J., Xu W., Zhao Z., Xu W. Z., Light D. R. and Morrissey, M. M. *Acta Crystallogr.* **D55**: 1395-1404 (1999).
- ⁸ Xu W., Davey D. D., Eagen, K., Guilford W. J., Ng H., Phillips G. B., Pinkerton M., Wu S. C., Linag A., Trinh L., Hinchman J., Post J., Sullivan M.E., Morrissey M. M. Am. Chem. Soc. Meet., Dallas, Texas, USA, Abstract #123.
- ⁹ Buckman, B. O., Mohan, R., Koovakkat, S., Liang, A., Trinh, L., Morrissey, M. M. *Bioorg. Med. Chem. Lett.* **8**, 2235-2240 (1998).