Journal of Fluorine Chemistry 129 (2008) 807–810

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

An enantioselective synthesis of (*S*)-4-fluorohistidine

Jan Hajduch, John C. Cramer, Kenneth L. Kirk *

Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, DHHS, Bethesda, MD 20892, United States

ARTICLE INFO

Article history:
Received 7 April 2008
Received in revised form 24 April 2008
Accepted 25 April 2008
Available online 2 May 2008

Keywords:
Schollkopf diasteroselective alkylation
Photochemical Schiemann reaction
Fluoroimidazole
Microwave reaction

ABSTRACT

We report a new synthesis of enantiomerically pure (*S*)-4-fluorohisitidine based on diastereoselective alkylation of MOM-protected 4-fluoro-5-bromomethyl imidazole using the Schöllkopf bis-lactim amino acid synthesis. Improvements in procedures for preparation of key intermediates are also described. (*S*)-4-Fluorohisitidine prepared by this new method was identical in all respects to material prepared by previous procedures.

Published by Elsevier B.V.

1. Introduction

Thirty-eight years have passed since we introduced the photochemical Schiemann reaction, a procedure that made possible the first synthesis of ring-fluorinated imidazoles [1]. The successful synthesis of (*S*)-2-fluorohistidine (**1**) and (*S*)-4-fluorohisitidine (**2**) (2-F-His, 4-F-His) (Fig. 1) was a particularly important application of this new fluorination procedure [2,3]. Subsequent biological studies on both isomers demonstrated that 4-F-His exhibited much lower activity or was lacking activity in many of the systems in which 2-F-His was quite potent. This biological activity of the latter in many cases apparently results from in vivo incorporation of the analogue into protein, and early results indicated that the 4-fluoro isomer was a poor substrate for protein biosynthetic enzymes. Accordingly, most of our subsequent work was done with the more active 2-fluoro isomer. Previously, 4-F-His was incorporated into ribonuclease *S*-peptide [4,5] and into ribonuclease A [6] using total synthesis. However, recent advances in molecular biology and biochemistry have made it possible to introduce a broader inventory of unnatural amino acids into proteins using protein biosynthetic enzymes. Thus, 4-F-His has recently been incorporated into PapD chaperone protein using biochemical techniques, albeit less efficiently than incorporation of 2-F-His [7]. As demonstrated with other amino acids such as fluorinated tryptophans, the ability to incorporate both regioisomers would provide protein and peptide analogues for NMR and functional studies that could probe more subtle consequences of analogue incorporation [8]. This renewed interest in 4-F-His has made re-synthesis necessary. We have taken this opportunity to apply more recent synthetic methodologies to this project and report herein the results of these efforts.

The original synthesis of 4-F-His was based on alkylation of sodium acetamidomalonate by 4-chloromethyl-5-fluoroimidazole to introduce the amino acid side chain. Hydrolysis and decarboxylation lead to the racemic amino acid. Resolution was accomplished by acylation and acylase-catalyzed kinetic resolution of the *N*-acetyl amino acid to afford the L-amino acid, but also leading to a 50% loss of material as the *N*-acetyl D-amino acid [2].

An alternative route involved *in situ* zinc metal reduction of side-chain protected 4-nitro-L-histidine followed by diazotization, irradiation, and deprotection. This procedure, although direct, suffered from poor yields [9]. We have now reinvestigated the synthesis of 4-F-His using an asymmetric approach. Other improvements in the reaction sequence are reported.

2. Chemistry

In order to obviate the resolution step required in our first synthesis of **2**, we replaced acetamidomalonate with the enantiopure nucleophile **3** (Fig. 1) developed by Schöllkopf [10] to effect a diastereoselective carbon–carbon bond formation. However, to make this practical we required appreciable amounts of ethyl 4-fluoroimidazole-5-carboxylate (**6**), the precursor for the alkylating agent. This in turn required a convenient source of ethyl 4-aminoimidazole-5-carboxylate (**5**) for use in the photochemical Scheimann reaction. This was available in our original synthesis

* Corresponding author. Tel.: +1 301 496 2619; fax: +1 301 402 4182.
E-mail address: kennethk@bdg8.niddk.nih.gov (K.L. Kirk).

0022-1139/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jfluchem.2008.04.011

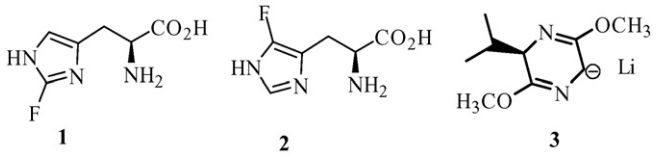


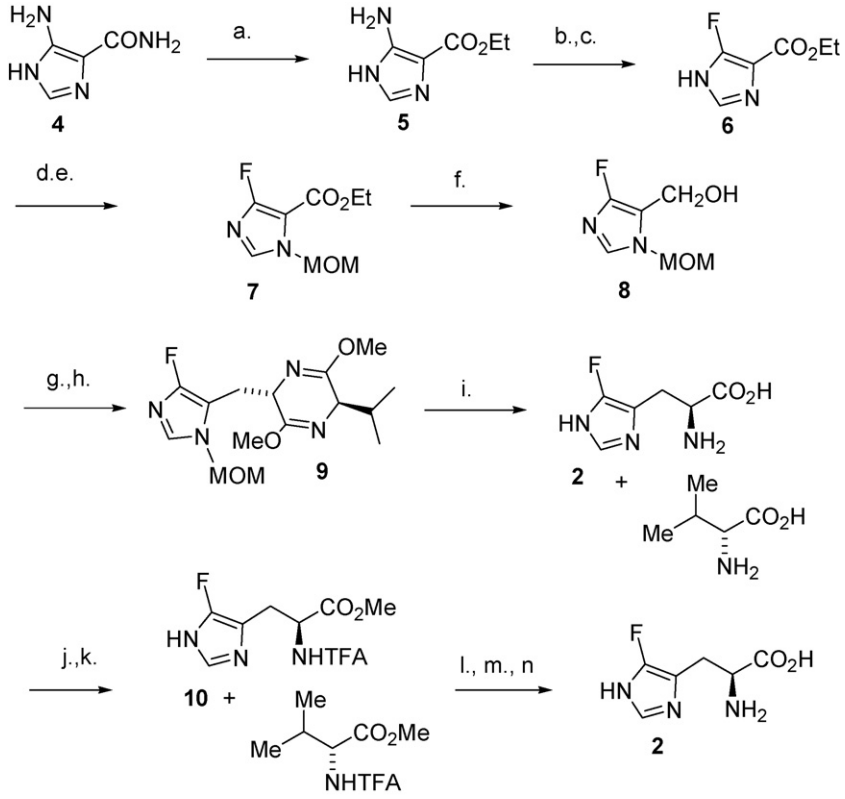
Fig. 1. Structures **1**,**2**,**3**.

through a tedious weeks-long acid-catalyzed ethanolysis of commercially available 5-aminoimidazole-4-carboxamide hydrochloride (**4**) [1,2]. A dramatic improvement in this first step of the synthesis was realized using microwave irradiation (Scheme 1). Thus, heating an ethanolic solution of 25 g of the amide and methane sulfonic acid in a microwave reactor at 155 °C produced 7–9 g of **5** in 40 min. Although the yield is approximately half that originally obtained, the time saved in initial production of material made up for the loss of product. Diazotization of **5** followed by photochemical decomposition to **6** proceeded as previously described [1,2]. However, the yield was improved from 39 to 53% by carrying out the photochemical step at −78 °C for 90 min [11]. We recently studied this reaction in ionic liquids and found that improved yields (63%) were offset by technical problems related to decomposition and viscosity of the ionic liquid [12].

In our previous synthesis, reduction of **6** gave highly polar 4-fluoro-5-hydroxymethyl imidazole in modest yield [2]. Separation of the highly polar product from lithium and aluminum salts appeared to be one of the causes of low recovery. In order to alleviate problems with product isolation, imidazole nitrogen protection was investigated. When the nitrogen was protected with a tosyl or trityl group, reduction of the ester group proved difficult. With the tosyl group no alcohol was obtained and starting material was recovered quantitatively. This presumably is a result of hydride reaction with the labile proton in the 2-position of the imidazole ring, but this was not investigated further. In contrast, using a MOM group allowed reduction to the alcohol in excellent yields. Thus, **6** was converted to **7** in CH_2Cl_2 with DIPEA at −12 °C by adding MOMCl dropwise and then allowing the reaction to return to room temperature over the course of 4 h (93–98% yield). Reduction of **7** with a 2.5–3 M excess of $LiAlH_4$ in ether (−78 °C → room temperature, 18 h) proceeded smoothly, and after soxhlett extraction of the aluminum oxide salts **8** was obtained in high yields (ca. 98%).

Side chain halogenation was carried out in the presence of CaH_2 to avoid loss of the MOM group [13]. Isolation of products of either bromination or chlorination reactions proved impossible, but LC/MS indicated only one product in the reaction mixture with masses consistent with either the bromo or chloro compound, respectively. Due to the difficulty encountered with isolation of this intermediate a one pot procedure was carried out for side chain elaboration. Bromination of the hydroxymethyl imidazole (**8**) was carried out by dropwise addition of a THF/PBr_3 (0.35 mol. eq.) solution to **8** in THF in the presence of CaH_2 (2 mol. eq.) at −78 °C. The reaction was allowed to gradually return to room temperature overnight (ca. 18 h) and then it was cooled to −100 °C for dropwise addition of the lithiated Schölkopf chiral reagent **3**. The reaction was maintained at −100° until completion (24–40 h) producing **9** in 63–72% yield (>98% d.e. after chromatography).

Cleavage of the dihydropyrazine ring to the methyl ester of (*S*)-4-fluorohisitidine in the presence of the MOM group proved difficult due to competing rates of hydrolysis. In the presence of 0.1 M HCl, hydrolysis resulted in a six-component mixture containing the methyl ester and acid forms of valine, and the methyl ester and acid forms 4-fluorohistidine with and without the



Scheme 1. *Conditions*: (a) EtOH, $MeSO_3H$, μW, 150 °C, 40 min; (b) $HBF_4/NaNO_2$; (c) −78 °C, *hν*, 90 min; (d) CH_2Cl_2, DIPEA, −12 °C; (e) MOMCl, −12 °C → RT, 18 h; (f) Et_2O, $LiAlH_4$, −78 °C → RT, 18 h; (g) THF, CaH_2, PBr_3, −78 °C → RT, 18 h; (h) **3**, −100 °C, 24–48 h; (i) Dowex-H^+ or 0.25 M HCl, 24–48 h, Dowex chromatography; (j and l) MeOH/HCl/MeOAc, μW, 150 °C, 10 min; (k) TFA_2O; (l) column chromatography; (m) 0.1 M NaOH; (n) Dowex.

imidazole ring MOM-protecting group. Because of this problem, complete cleavage directly to the amino acid was carried out with aqueous HCl. The resulting mixture of (*S*)-4-fluorohisitidine and valine was converted to the *N*-trifluoroacetyl methyl ester derivatives by sequential treatment with methanolic HCl and trifluoroacetic anhydride. *N*-Trifluoroacetyl-(*S*)-4-fluorohisitidine methyl ester **10** was separated by flash chromatography and purified. This was then saponified and isolated as the free amino acid **2** by Dowex chromatography and lyophillization. This material was identical to that made by previous procedures [2,9].

The spectral and data physical, including rotation, of **2** prepared by this route were in complete agreement with authentic compound characterized from previous syntheses. The use of microwave irradiation in preparation of starting material and improvement of the key reduction step are important advances from this synthesis.

3. Experimental

3.1. General

All the reagents were from commercial sources and used without further purification. NMR spectra were run in $CDCl_3$ on a Varian Gemini 300 MHz spectrometer. Mass spectra were determined using a Jeol SX-102 instrument.

3.2. Ethyl 4-aminoimidazole-5-carboxylate (5)

To a solution of 25 g (0.154 mol) of 4-aminoimidazole-5-carboxamide hydrochloride in 180 mL of ethanol was added 60 mL of methane sulfonic acid. After initial mixing, a lavender precipitate formed and the microwave container was sealed. The mixture was subjected to microwave radiation (variable voltage) to 155–165 °C (the temperature was adjusted to keep vessel pressure under 21 bar). Upon cooling, the precipitate that formed in the reaction vessel was removed by vacuum filtration and was washed with several aliquots of ethanol. The precipitate was dissolved in water and the pH was checked and adjusted to neutrality. The filtrate was neutralized with 6 M NaOH and the solvent was removed by rotary evaporation. This material was combined with the solution of salts originally precipitated from the aqueous solution above and extracted multiple times (≥5 extraction) with allotments of EtOAc (≥200 mL). The combined EtOAc extracts were washed (H_2O, brine) and then dried over sodium sulfate. Removal of the solvent produced **5** as a white solid. This was purified either by fractional crystallization (EtOAc/ethanol) or column chromatography (DCM/MeOH w/0.1% Et_3N). 4-Aminoimidazole-5-carboxylate ethyl ester (**5**) was obtained as colorless crystals in yields ranging from 7 to 9 g (29–37%) in multiple runs. This was identical in all respects to material obtained previously using thermal ethanolysis [2].

3.3. Ethyl 4-fluoroimidazole-5-carboxylate (6)

A solution of 4.98 g (32.1 mmol) of **5** in 100 mL of 48% HBF_4 was cooled to −12 °C and a solution 2.88 g (41.7 mmol) of $NaNO_2$ dissolved in a minimal amount of water was added dropwise with stirring. After 15 min, the reaction mixture was transferred to a 200 mL photochemical reaction flask and filled with cold HBF_4. The solution then was cooled to −78 °C with dry ice/acetone and irradiated for 90–120 min using a Hanovia medium pressure ultraviolet lamp and quartz immersion well fitted with a corex filter. The resulting solution was neutralized (50% NaOH) at −78 °C, and after warming to room temperature was extracted with aliquots of EtOAc (4–6 times with 200–300 mL per extraction). After drying (Na_2SO_4) and removal of the solvent, flash chromatography (hexane/EtOAc, 75:25 → 25:75) produced 2.74 g (54% yield) of **6**. This was identical in all respects with material prepared previously [1,2].

3.4. 5-Fluoro-1-methoxymethyl-1H-imidazole-4-carboxylic acid ethyl ester (7)

A solution of **6** (1.70 g, 11.8 mmol) in 100 mL of CH_2Cl_2 was chilled to −12 °C and 2.5 mL (15 mmol) of DIPEA was added. After 15 min, MOMCl (1.2 mL, 15.7 mmol) was added dropwise. The reaction was kept at −12 °C for 1 h and then was allowed to warm to room temperature for 30 min. The reaction was poured into water (200 mL) and extracted with CH_2Cl_2 (3× 100 mL). After washing with brine and drying (Na_2SO_4), removal of the solvent produced 2.43 g (>100% yield) of **7** as a clear light yellow oil. This was of sufficient purity to use in the next step, but a short plug of silica could be used to remove the minor impurities to give 2.36 g (98% yield) of **7**. In general, yields of ≥98% were obtained in this reaction. ^1H ($CDCl_3$): 1.38 (t, 3H, CH_3, $^3J_{HH}$ = 7.2), 3.37 (s, 3H, CH_3), 4.36 (q, 2H, $^3J_{HH}$ = 7.2), 5.59 (d, 2H, CH_2, $^4J_{HF}$ = 0.9), 7.43 (d, 1H, imid. C2–H, $^4J_{HF}$ = 1.5). ^{13}C ($CDCl_3$): 14.26 (s, CH_3), 56.73 (s, CH_3), 60.88 (s, CH_2), 78.15 (s, CH_2), 102.88 (d, imid. C4, $^2J_{CF}$ = 28.7), 135.42 (d, imid. CH, $^3J_{CF}$ = 15.4), 159.17 (d, CO, $^3J_{CF}$ = 5.1), 159.66 (d, imid. CF, $^1J_{CF}$ = 253.3). ^{19}F ($CDCl_3$): −113.88 (s, 1F). HR-MS (ESI): for $C_8H_{12}N_2O_3F$ calculated 203.0832 (M+1), found 203.0832 (M+1). Anal. Calcd. for $C_8H_{11}N_2O_3F$: C, 47.52; H, 5.48. Found: C, 47.46; H, 5.50.

3.5. (5-Fluoro-1-methoxymethyl-1H-imidazol-4-yl)-methanol (8)

A solution of **7** (2.14 g, 10.6 mmol) in ether (35 mL) under a N_2 atmosphere was cooled to −78 °C. To this was added dropwise a solution of 44 mL (22 mmol) of 0.5 M LAH in diglyme. The reaction was allowed to gradually warm to room temperature over night. Cold water saturated with CO_2 then was added dropwise to quench the reaction. The aluminum oxide salts were removed by filtration and were subjected to soxhlet extraction with EtOAc for 3 h to separate the additional **8** from the salts. The original filtrate was extracted with EtOAc and combined with the solution from the soxhlet extraction. After drying, removal of EtOAc followed by column chromatography (hexane/EtOAc, 1:1) produced 1.60 g (10 mmol, 95% yield) of **8**. ^1H ($CDCl_3$): 3.31 (d, 3H, CH_3, $^4J_{HF}$ = 2.4), 3.64 (s, 1H, OH), 4.62 (bs, 2H, CH_2), 5.29 (d, 2H, CH_2, $^4J_{HF}$ = 1.2), 7.19 (d, 1H, imid. C2–H, $^4J_{HF}$ = 1.5). ^{13}C ($CDCl_3$): 51.16 (d, CH_2, $^3J_{CF}$ = 3.7), 56.25 (s, CH_3), 77.44 (s, CH_2), 109.38 (d, imid. C4, $^2J_{CF}$ = 33.5), 131.26 (d, imid. CH, $^3J_{CF}$ = 16.0), 154.62 (d, imid. CF, $^1J_{CF}$ = 238.4). ^{19}F ($CDCl_3$): −137.13 (s, 1F). HR-MS (ESI): for $C_6H_{10}N_2O_2F$ calculated 161.0726 (M+1), found 161.0733 (M+1). Anal. Calcd. for $C_6H_9N_2O_2F$: C, 45.00; H, 5.66. Found: C, 44.65; H, 5.75.

3.6. 2-(5-Fluoro-1-methoxymethyl-1H-imidazol-4-ylmethyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-pyrazine (9)

To a solution of **8** (1.66 g, 10.4 mmol) and CaH_2 (895 mg, 21.3 mmol) in 15 mL of THF cooled to −78 °C under N_2 was added a solution of PBr_3 (1.05 g, 3.89 mmol) in 10 mL of THF. The reaction was allowed to gradually warm to room temperature overnight. TLC confirmed completion of the reaction at this time and the solution was cooled to −100 °C. A solution of lithiated **3** was prepared in the usual manner. Thus, 6.96 mL (17 mmol) of a 2.5 M solution of *n*BuLi in hexanes was added dropwise to a solution of the pyrazine **3** (3.24 g, 17.6 mmol) in 15 mL of THF cooled to −78 °C. After stirring at −78 °C for 30 min, the solution of **3** was cooled to −100 °C. The resulting lithiated pyrazine **3** was then

transferred dropwise via cannula to the brominated derivative of **8**. The reaction was maintained at −100 °C until TLC indicated no starting material remained (24–48 h). After warming to room temperature, the reaction was quenched by the addition of cold water. After removal of THF by rotary evaporation, the aqueous solution was extracted with EtOAc (3× 100 mL). The extracts were washed with brine, dried (Na_2SO_4) and the solvent was removed to give 2.44 g (72%) of crude **9** with d.e. 91%. Flash chromatography with hexane/EtOAc (3:1 → 1:1) gave 2.24 g (66%) with d.e. >98%. $[\alpha]_D = -37.5^\circ$ ($c = 1$, chloroform). ^1H ($CDCl_3$): 0.83 (d, 3H, CH_3, $^3J_{HH} = 6.6$), 0.96 (d, 3H, CH_3, $^3J_{HH} = 7.2$), 2.13–2.23 (m, CH), 3.17 (dd, 1H, CH_2, $^2J_{HH} = 15.6$, $^3J_{HH} = 6.9$), 3.24 (s, 3H, CH_3), 3.34 (dd, 1H, CH_2, $^2J_{HH} = 15.3$, $^3J_{HH} = 4.2$), 3.69 (s, CH_3), 3.73 (m, 1H), 4.25 (ddd, 1H, CH, $^3J_{HH} = 6.6$, $^3J_{HH} = 4.5$, $^5J_{HF} = 2.4$), 5.13 (dd, 1H, CH_2, $^2J_{HH} = 11.1$, $^4J_{HF} = 1.8$), 5.42 (d, 1H, CH_2, $^2J_{HH} = 10.8$), 7.15 (d, 1H, imid. C2–H, $^4J_{HF} = 1.5$). ^{13}C ($CDCl_3$): 16.11 (s, CH_3), 18.24 (s, CH_3), 26.20 (d, CH_2, $^3J_{CF} = 4.0$), 53.09 (s, CH), 55.77 (s, CH), 57.55 (s, CH_3), 58.71 (s, CH_3), 77.23 (s, CH_2), 106.00 (d, imid. C4, $^2J_{CF} = 34.7$), signal of imid. CH hidden in noise, 155.84 (d, imid. CF, $^1J_{CF} = 235.9$), 159.73 (s, C═), 170.80 (s, C═). ^{19}F ($CDCl_3$): −135.89 (s, 1F). HR-MS (ESI): for $C_{15}H_{24}N_4O_3F$ calculated 327.1832 (M+1), found 327.1846 (M+1).

3.7. 3-(5-Fluoro-1H-imidazol-4-yl)-2-(2,2,2-trifluoro-acetylamino)-propionic acid methyl ester (10)

A solution of **9** (112.8 mg, 0.3456 mmol) in 10 mL of 0.25 M aqueous HCl was stirred at room temperature of 24–48 h until TLC showed complete consumption of starting material. Removal of solvent by rotary evaporation produced 142.5 mg of a crystalline mixture of products, including the hydrochloride salts of **2** and valine. This mixture was dissolved in methanolic HCl (prepared by addition of AcCl to anhydrous methanol) and subjected to microwave irradiation at 140 °C for 10 to 20 min. The methanol was removed and the residue was treated overnight with 10 mL of trifluoroacetic anhydride. After removal of trifluoroacetic anhydride, flash chromatography (hexane/EtOAc, 1:1 → 1:2) of the residue produced 93.4 mg (95% yield) of **10**. $[\alpha]_D = -2.7^\circ$ ($c = 1$, acetone). ^1H (CD_3OD): 3.07 (dd, 1H, CH_2, $^2J_{HH} = 15.3$, $^3J_{HH} = 9.0$), 3.20 (dd, 1H, CH_2, $^2J_{HH} = 15.3$, $^3J_{HH} = 5.4$), 3.76 (s, 3H, CH_3), 4.72 (dd, 1H, CH, $^3J_{HH} = 9.0$, $^3J_{HH} = 5.7$), 7.22 (d, 1H, imid. C2–H, $^4J_{HF} = 1.5$). ^{13}C (CD_3OD): 25.50 (d, CH_2, $^3J_{CF} = 4.0$), 53.38 (s, CH_3), 53.61 (d, CH, $^4J_{CF} = 1.1$), 103.96 (d, imid. C4, $^2J_{CF} = 34.7$), 117.41 (q, CF_3, $^1J_{CF} = 286.8$), 129.79 (d, imid. CH, $^3J_{CF} = 15.8$), 155.95 (d, imid. CF, $^1J_{CF} = 232.7$), 158.98 (q, CO, $^3J_{CF} = 37.8$), 171.45 (s, CO). ^{19}F (CD_3OD): −75.65 (s, 3F, CF_3), −144.05 (s, 1F, imid. CF). HR-MS (ESI): for $C_9H_{10}N_3O_3F_4$ calculated 284.0658 (M+1), found 284.0657 (M+1). Anal. Calcd. for $C_9H_9N_3O_3F_4$: C, 38.17; H, 3.20. Found: C, 38.17; H, 3.19.

3.8. (S)-4-Fluorohisitidine (2)

A solution of **10** (90 mg, 0.32 mmol) in 16 mL of 0.1 M NaOH was stored at 4 °C in a refrigerator overnight. The solution was then added to a column of Dowex 50W8X (acid form) and eluted first with water until the eluant was neutral. The free amino acid was washed from the column with a solution of 1:4 aqueous ammonium hydroxide. Lyophylization of the fractions containing product produced 54.1 mg (91% yield) of **2**. This was identical in all respects with material prepared previously [2].

Acknowledgement

This research was supported by the intramural research funds of NIDDK.

References

[1] L.A. Cohen, K.L. Kirk, J. Am. Chem. Soc. 93 (1971) 3060–3061.
[2] K.L. Kirk, L.A. Cohen, J. Am. Chem. Soc. 95 (1973) 4619–4624.
[3] K.L. Kirk, W. Nagai, L.A. Cohen, J. Am. Chem. Soc. 95 (1973) 8389–8392.
[4] I.M. Chaiken, H.C. Taylor, in: R. Walter, J. Meienhofer (Eds.), Proceedings of the 4th American Peptide Symposium in Peptides: Chemical, Structure & Biology, Ann Arbor Sci., Ann Arbor, MI, 1975, pp. 1013–1020.
[5] H.C. Taylor, I.M. Chaiken, J. Biol. Chem. 252 (1977) 6991–6994.
[6] D.J. Jackson, J. Burnier, C. Quan, M. Stanley, J. Tom, J.A. Wells, Science 266 (1994) 243–247.
[7] J.F. Eichler, J.C. Cramer, K.L. Kirk, J.G. Bann, ChemBiochem 6 (2005) 2170–2173.
[8] R. Gosh, E.A. Pratt, V. Simplaceanu, C. Ho, Biochemistry 26 (1987) 549–556.
[9] K.L. Kirk, L.A. Cohen, J. Org. Chem. 38 (1973) 3647–3648.
[10] U. Schöllkopf, Tetrahedron 39 (1983) 2085–2091.
[11] See also K. Takahashi, K.L. Kirk, L.A. Cohen, J. Org. Chem. 49 (1984) 1951–1954.
[12] J. Heredia-Moya, K.L. Kirk, J. Fluorine Chem. 128 (2007) 674–678.
[13] E.J. Corey, D.E. Cane, L. Libit, J. Am. Chem. Soc. 93 (1971) 7016–7021.