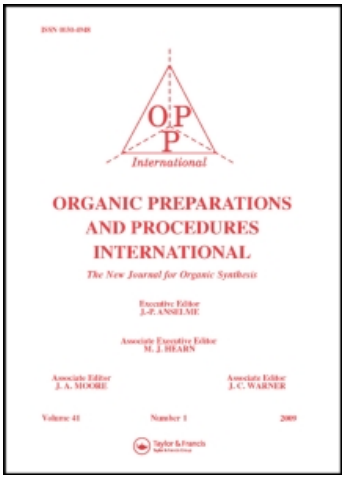


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# SYNTHESIS OF 5,5'DIFLUORO-BAPTA-AM, A USEFUL *IN VIVO* PROBE FOR CALCIUM DETERMINATION

Philip E. Morris Jr.[ab]
[a] Department of Chemistry, University of Alabama at Birmingham, Birmingham, AL [b] BioCryst Pharmaceuticals, Birmingham, AL

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# SYNTHESIS OF 5,5'-DIFLUORO-BAPTA-AM, A USEFUL *IN VIVO* PROBE FOR CALCIUM DETERMINATION

Philip E. Morris, Jr.†

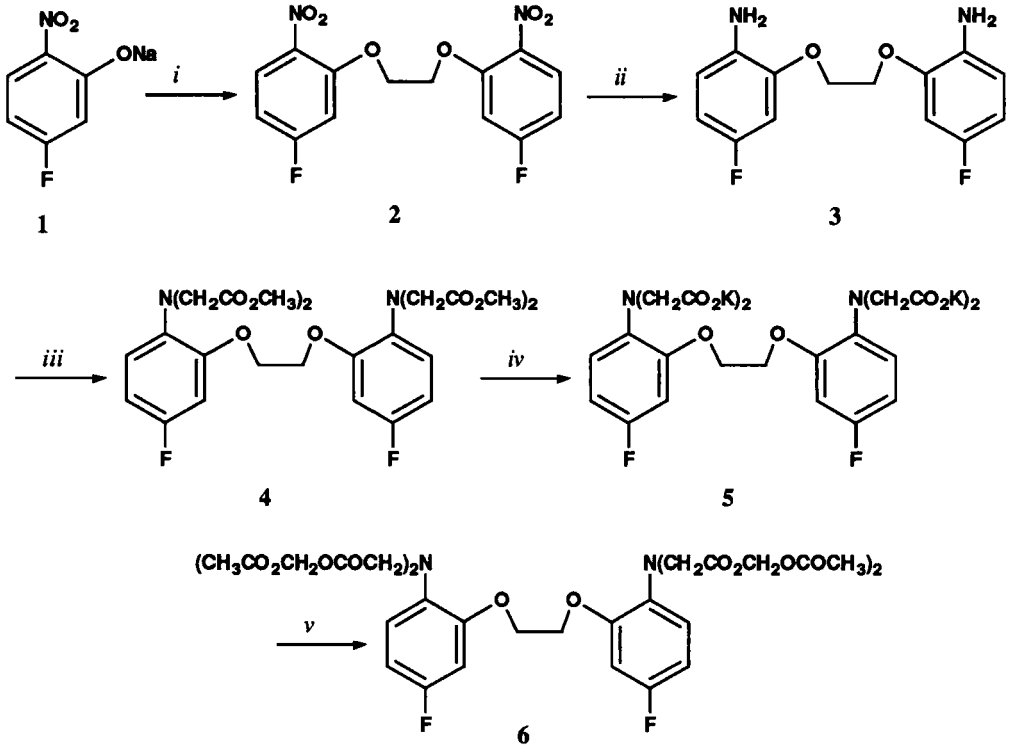
*University of Alabama at Birmingham*
*Department of Chemistry, PSB - 227, Birmingham, AL 35294*



In recent years the importance of cytosolic free calcium ($Ca^{+2}$) has begun to emerge. It is now recognized that $Ca^{+2}$ is involved in cell regulation, metabolism, and may also serve as a second messenger. As a consequence, there is increasing demand for noninvasive analytical techniques for measuring intracellular calcium in intact viable tissues. A number of fluorescent probes have been synthesized for detecting free $Ca^{+2}$ using spectrofluorometric techniques.[1] These probes selectively chelate $Ca^{+2}$ and are extremely sensitive, however, many of these fluorescent compounds are limited to the detection and quantification of surface $Ca^{+2}$. Prominent in this class are 1,2-*b*is(o-*a*mino*p*henoxy)ethane-*N*,*N*,*N'*,*N'*-*t*etra*a*cetic acid (BAPTA) and its derivatives.[2] BAPTA and its derivatives are available in two forms. The tetraanion (K salt) form, which is cell impermeable can be used for extracellular $Ca^{+2}$ determinations. Cell or tissue loading requires the use of an acetoxymethyl ester (AM) protected form which provides sufficient lipophilicity for cell penetration. Once into the cell, the acetoxymethyl ester group is enzymatically cleaved by an esterase to give the tetraanion which is cell impermeable (*i.e.* trapped in the cell) and acts as the active chelator. For NMR detection, fluorine has been incorporated into BAPTA. Of the fluorinated analogs available, 5,5'-difluoro-BAPTA (5FBAPTA) is the most widely used probe for studying cytosolic free $Ca^{+2}$ by $^{19}F$ NMR[3]. It has high selectivity for $Ca^{+2}$, is not particularly pH sensitive, and is available as the salt form or protected as its acetoxymethyl ester. At present, fluorinated BAPTA analogs, in particular 5FBAPTA-AM, are the only probes available which gives the *volume averaged* $Ca^{+2}$ concentration. In addition, determinations with 5FBAPTA are not sensitive to cells which have a high natural background fluorescence. These properties combine to make 5FBAPTA a very versatile and attractive biological probe for elucidating the role of cytosolic $Ca^{+2}$.

As part of a collaborative study examining free intracellular $Ca^{+2}$ in perfused rat heart, we required gram quantities of 5FBAPTA-AM (**6**). Although the compound is available commercially,[4] studies requiring more than milligram quantities are cost prohibitive. A review of the literature revealed that a number of similar 5-substituted BAPTA analogs have been synthesized.[2] However, synthesis of the 5-fluorinated analog has not been previously described. Moreover, the step involving

conversion to the acetoxymethyl ester has not been reported for the analogs, with the authors relying on a commercial firm to carry out the last step for them.[5] The present paper now describes a convenient synthesis of 5FBAPTA (**5**) and its final conversion to the cell-permeable acetoxymethyl ester form (5FBAPTA-AM, **6**).




*i*) $BrCH_2CH_2Br$, DMF; *ii*) $H_2$, Pd-C; *iii*) $BrCH_2CO_2CH_3$, $CH_3CN$; *iv*) KOH, MeOH, $H_2O$; *v*) $CH_3CO_2CH_2Br$, $CH_3CN$.

Commercially available 5-fluoro-2-nitrophenol was converted to the corresponding sodium salt **1** in aqueous methanol with sodium hydroxide. At reflux temperature, **1** was reacted with 1,2-dibromoethane in DMF. After 2 hrs, the original bright orange color of the reaction mixture was discharged with concurrent formation of solid NaCl. The coupled product **2** was collected by pouring the mixture onto ice-water and removing the product by vacuum filtration. It was then necessary to recrystallize **2** from 95% aqueous ethanol prior to the reduction step. Reduction of the nitro group of **2** was effected with $H_2$/Pd-C in 95% ethanol and generally required *ca.* 20 hrs at 45-50 psi. The progress of the reduction is easily monitored by observing the discharge of the greenish-yellow color of **2** and its replacement with a white solid. Removal of the Pd-C catalyst at this stage gave a material which was rapidly converted to a compound which was deep blue. Most likely, this latter material represents some oxidation by-product of the amine and the Pd-C acts as an *in situ* reducing agent (or anti-oxidant) and is required to maintain the integrity of the amine product. Alkylation of **3** with methyl bromoacetate in acetonitrile gave **4** which was purified by column chromatography. Saponification of the tetraester **4**

with aqueous methanol containing 4 equivalents of KOH gave the tetrapotassium carboxylate **5** which was sufficiently pure for the last step. The final conversion to **6** was effected by the careful esterification of **5** with bromomethyl acetate to give the acetoxymethyl ester. The final compound was purified by column chromatography and the resulting syrup was then crystallized with absolute methanol. It was found necessary to purify crude **6** to near homogeneity prior to crystallization.

## EXPERIMENTAL SECTION

All reagents and solvents were of the highest available purity. Dimethylformamide was distilled from BaO prior to use and acetonitrile was distilled from $P_2O_5$. Thin layer chromatography (TLC) analyses were performed on Kieselgel aluminum backed silica gel 60 $F_{254}$ plates (0.2 mm) obtained from E. Merck and were visualized using an ultraviolet light (254 nm) or $I_2$. Column chromatography was achieved with silica gel from Baker. Melting points were recorded in capillary tubes on a Mel-Temp apparatus and are uncorrected. All $^1H$ NMR spectra were recorded at 300 MHz with a Nicolet Fourier Transform Spectrometer in $CDCl_3$ or DMSO-$d_6$, unless otherwise noted. Resonances are reported downfield from internal tetramethylsilane. Multiplicities are reported as follows: s, singlet; d, doublet; dd, doublet of doublets; Ψt, apparent triplet (*i.e.* $J_{ab} = J_{bc}$); q, quartet; m, multiplet. IR spectroscopy was performed with Perkin-Elmer 1310 instrument. Micro-analyses were performed by Atlantic Microlabs, P. O. Box 2288, Norcross, GA 30091-9990.



**Sodium 5-fluoro-2-nitrophenoxide (1).**- 5-Fluoro-2-nitrophenol (15.7 g, 0.1 mol) was suspended in a solution of water (25 mL) and methanol (15 mL), containing sodium hydroxide (4.0 g, 0.1 mol). The mixture immediately developed a bright orange color and was stirred for 30 min. The solvents were removed under diminished pressure and the remaining water removed by azeotroping with toluene to give 17.9 g of **1** (100%) as a bright, orange-red, flocculent material, mp. > 280°. $^1H$ NMR (DMSO-$d_6$): δ 7.76 (Ψt, $J$ = 7.8, Ar*H*), 6.13 (dd, $J$ = 13.5, $J$ = 2.7, Ar*H*), 5.89 (m, Ar*H*).

**1,2-bis(5-Fluoro-2-nitrophenoxy)ethane (2).**- Sodium salt **1** (3.58 g, 20 mmol) and 1,2-dibromoethane (0.97 mL, 11.2 mmol) were heated to reflux in DMF (15 mL) for 2 hrs. The reaction mixture was cooled and then poured onto crushed-ice (*ca.* 100 mL). The resulting mustard colored product was collected and the crude product recrystallized from 95% ethanol to give **2** (65%) as fine yellow-green crystals, mp. 162-164.5°. $^1H$ NMR ($CDCl_3$): δ 7.95 (dd, $J$ = 9.0, $J$ = 6.0, Ar*H*), 6.93 (dd, $J$ = 9.0, $J$ = 2.7, Ar*H*), 6.79 (m, Ar*H*) 4.54 (s, ArOC$H_2$).

*Anal.* Calcd. for $C_{14}H_{10}F_2N_2O_6$: C, 49.42; H, 2.96; N, 8.23. Found C, 49.52, H, 2.96, N, 8.26

**1,2-bis(5-Fluoro-2-aminophenoxy)ethane (3).**- Compound **2** (5.0 g, 14.7 mmol) was suspended in 95% ethanol containing 10% Pd-C (250 mg). The mixture was hydrogenated at 50 psi for 24 hrs in a Parr shaker. After absorption of the theoretical amount of hydrogen, the yellow color of the starting material was discharged. The mixture, including the catalyst, was transferred to a round bottom flask and the solvent was removed under diminished pressure to give **3** (4.1 g, 14.7 mmol) in quantitative yield. The resulting amine product containing the hydrogenation catalyst was used without purification.

**1,2-bis(5-Fluoro)-[2-[bis[(methoxycarbonyl)methyl]amino]phenoxy]ethane (4).**- Amine **3** (2.5 g, 8.93 mmol), 1,8-bis(dimethylamino)naphthalene (Proton Sponge®, 9.6 g, 44.7 mmol), sodium iodide

(1 g), and methyl bromoacetate (4.9 mL, 51.8 mmol) were refluxed in dry acetonitrile (20 mL) under nitrogen for 24 hrs. The dark mixture was cooled, and vacuum filtered to remove solids which were washed thoroughly with chloroform. Evaporation of the combined organic phases gave a dark solid which was preabsorbed on silica gel prior to column chromatography. Elution with ether-toluene (1:1) gave a slightly colored residue which was sufficiently pure for the next step. An analytical sample was prepared by recrystallization from 95% ethanol, mp. 115-116°. $^1$H NMR ($CDCl_3$): δ 6.84 (q, $J$ = 8.7, Ar*H*), 6.61 (m, Ar*H*), 4.27 (s, ArOC*H*$_2$), 4.09 (s, NC*H*$_2$), 3.58 (s, C*H*$_3$).
*Anal.* Calcd. for $C_{26}H_{30}F_2N_2O_{10}$: C, 54.93; H, 5.32; N, 4.93. Found C, 54.87; H, 5.36, N, 4.89

**Tetrapotassium 1,2-bis(5-Fluoro-2-aminophenoxy)ethane-N,N,N',N'-tetraacetate (5).**- The ester **4** (1.0 g, 1.8 mmol) was refluxed for 1 hr with KOH (0.42 g, 7.4 mmol) in a solution of water (10 mL) and methanol (25 mL). Removal of the solvent gave crystalline **5** (100%) which was used without further purification, mp. > 280°. $^1$H NMR ($D_2O$): δ 6.90 (m, Ar*H*), 6.71 (m, Ar*H*), 4.40 (s, ArOC*H*$_2$), 3.71 (s, NC*H*$_2$).

**1,2-bis[(5-Fluoro)-2-[bis[(acetoxymethoxy)carbonyl)methyl]amino]phenoxy]ethane (6).**- The tetrapotassium salt **5** (1.0 g, 1.5 mmol) was suspended in dry acetonitrile (15 mL) under nitrogen. To the stirred solution was then added bromomethyl acetate (0.77 mL, 7.8 mmol). The reaction was heated to reflux for 24 hrs and then allowed to cool. The mixture was filtered to remove solids, which were washed thoroughly with acetonitrile. The organic phases were combined and the solvent removed to give 1.8 g of crude **6**. The resulting oil was chromatographed on silica gel to give a pale yellow syrup. The syrup was crystallized from anhydrous methanol to give **6** as colorless needles, mp. 67-68° (auth. mp. 65-67°). IR 1770 cm$^{-1}$ (C=O), 1270 cm$^{-1}$ (CN), 1150 cm$^{-1}$ (C-O); $^1$H NMR (DMSO-$d_6$): δ 6.93 (dd, $J$ = 2.4, $J$ = 10.5, Ar*H*), 6.81 (dd, $J$ = 6.3, $J$ = 9.0), 6.68 (m, Ar*H*), 5.57 (s, OC*H*$_2$O), 4.27 (s, ArOC*H*$_2$), 4.12 (s, NC*H*$_2$), 2.02 (s, C*H*$_3$).
*Anal.* Calcd. for $C_{34}H_{18}F_2N_2O_{18}$: C, 51.00; H, 4.78; N, 3.50. Found C, 51.10; H, 4.79; N, 3.46



## REFERENCES

† Present address: BioCryst Pharmaceuticals, 2190 Parkway Lake Drive, Birmingham, AL 35244.

1. G. Grynkiewicz, M. Poenie and R. Y. Tsien, *J. Biol. Chem.*, **260**, 3440 (1985).
2. R. S. Tsien, *Biochemistry*, **19**, 2396 (1980).
3. G. A. Smith, R. T. Hesketh and J. C. Metcalfe, *Proc. Natl. Acad. Sci. USA*, **80**, 7178 (1983).
4. Molecular Probes, P. O. Box 22010, Eugene, OR 97403-0414.
5. L. A. Levy, E. Murphy, and R. E. London, *Am. J. Physiol.*, **252**, (Cell Physiol. 21), C441 (1987).

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