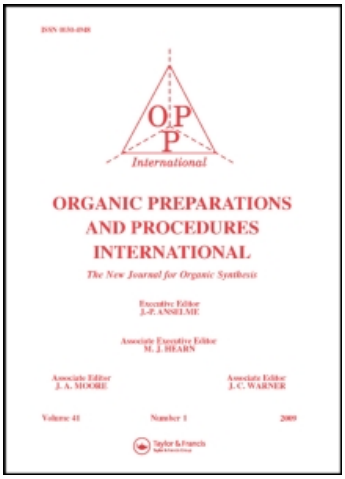


Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

A PRACTICAL METHOD FOR THE SYNTHESIS OF PHENOPHTHALEIN SPIROLACTAMS

Maciej Adamczyk[a]; Jonathan Grote[a]
[a] Divisional Organic Chemistry Research (D9NM) Abbott Diagnostics Division, Abbott Luborotories, Abbott Park, IL

To cite this Article Adamczyk, Maciej and Grote, Jonathan(2001) 'A PRACTICAL METHOD FOR THE SYNTHESIS OF PHENOPHTHALEIN SPIROLACTAMS', Organic Preparations and Procedures International, 33: 1, 95 — 100
To link to this Article: DOI: 10.1080/00304940109356580
URL: http://dx.doi.org/10.1080/00304940109356580

21. T. D. Fulmer, L. P. Dasher, B. L. Bobb, J. D. Wilson, K. L. Sides and C. F. Beam, *J. Heterocycl. Chem.*, **17**, 799 (1980).

22. M. J. Livingston, M. F. Chick, E. O. Shealy, D. J. Park, T. D. Fulmer and C. F. Beam, *ibid.*, **19**, 215 (1982).

23. C. F. Beam, H. L. Hall, A. M. Huff, R. C. Tummons and S. A. O'Grady, *ibid.*, **21**, 1897 (1984).

24. P. Mirone and M. Vampiri, *Atti. Accad. Nazl. Lincei., Rend. Classe Sci. Fis., Mat e Nat.*, **12**, 583 (1952); *Chem. Abstr.*, **46**, 9423 (1952).

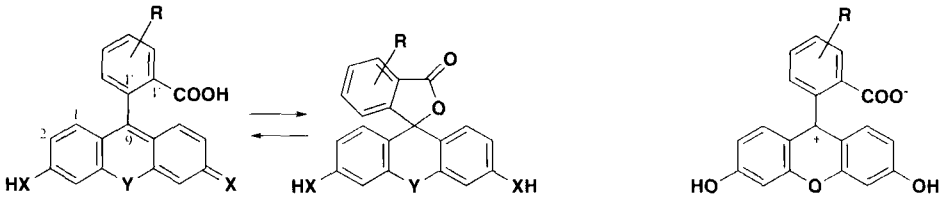
****

A PRACTICAL METHOD FOR THE SYNTHESIS OF PHENOPHTHALEIN SPIROLACTAMS

Submitted by Maciej Adamczyk* and Jonathan Grote
(7/12/2000)

Divisional Organic Chemistry Research (D9NM)
Abbott Diagnostics Division
Abbott Laboratories, Abbott Park, IL 60064

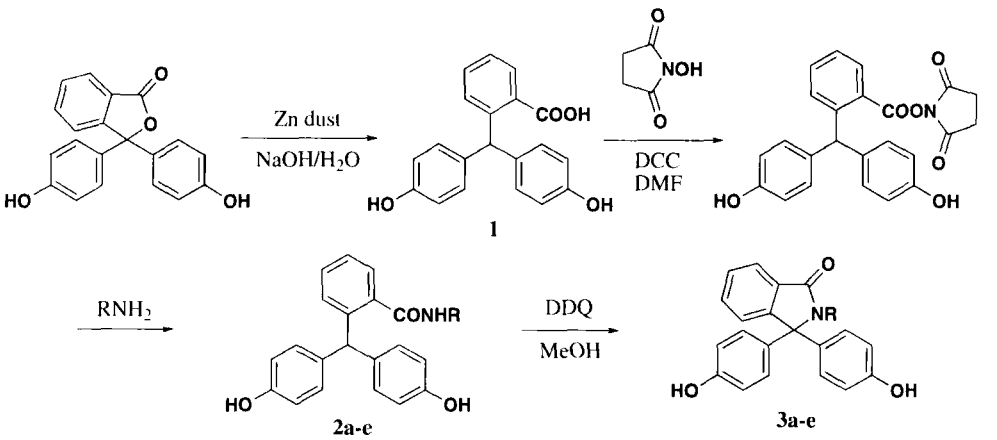
Since their synthesis in the late 1800s, various dyes have been utilized in many biotechnological applications.[1] Of these dyes, the fluoresceins are the most commonly used labels due to their solubility in aqueous buffers and high fluorescence quantum yield at physiological pH,[2] although the rhodamines are prized for their great photostability, pH insensitivity over a broad range (low to neutral pH), and the ability of their fluorescence characteristics to be tailored for a particular application by changing ring and nitrogen substituents.[2] Traditionally, these dyes have been viewed to exist in two pH dependent forms: a highly colored open, quinone form and a nearly colorless, closed, spirolactone form (see below),[3] although more recently a third zwitterionic structure has been postulated for fluorescein in aqueous solution.[4,5]



(rhodamine: X = NH, RN, Y = O; fluorescein: X = Y = O

We have recently reported a new method for the synthesis of a novel family of spirolactams of fluorescein[6] and rhodamines,[7] which can be used to prepare masked fluorescent conjugates. A desirable extension of this work would include a study of phenolphthalein spirolactams, the synthesis of which has not been previously described. We report here a simple, practical synthesis of phenolphthalein spirolactams, useful for the synthesis of a variety of derivatives.

Initially, we hypothesized that the synthesis of the phenolphthalein analogs could be achieved by using the procedure reported for synthesis of fluorescein and rhodamine spirolactams, *i.e.*, by reaction of their reported esters with primary amines. However, a search of the literature revealed no reports of the preparation of pure, stable phenolphthalein esters. Furthermore, attempts to prepare phenolphthalein esters by a variety of different methods failed. It was then decided to reduce phenolphthalein to its free acid, which would be more amenable to activation and amide formation. Subsequent reoxidation would provide the desired spirolactam. Phenolphthalein was therefore reduced by a known procedure utilizing zinc dust in boiling potassium hydroxide solution to yield dihydrophenolphthalein (**1**).[8] The free carboxyl group of the non-protected dihydrophenolphthalein was then activated with N-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide to form the activated ester, which reacted straightforwardly with a variety of different amines. The resulting amides **2a-e** all exhibited singlets near δ 6.0 in ^{1}H NMR (for the C9 *H*) and a ^{13}C resonance near 49 ppm (for the C9 *C*-H). The amides were then oxidized with DDQ, providing the desired phenolphthalein spirolactams **3a-e** in good yield, as evidenced by a ^{13}C resonance near 70 ppm (C9 *C*-N) and disappearance of the 6.0 ppm singlet. Although dihydrophenolphthalein hydrazide and dihydrophenolphthalein hydroxyamide could also be synthesized, treatment of the hydrazide with DDQ resulted in formation of a hydrazone with the DDQ, and treatment of the hydroxyamide with DDQ produced a six-membered spirooxylactam.[10]



a) R = H; b) R = CH_3; c) R = CH_2COOH; d) R = $CH_2CH_2CH_2NH_2$; e) R = $CH_2CH_2CH_2OH$

In conclusion, we have developed a simple, practical synthesis of phenolphthalein spirolactams. The compounds are produced as colorless solids in good three-step yields.

EXPERIMENTAL SECTION

All reagents were purchased from Aldrich and used without further purification. Solvents used were of HPLC grade and used without further purification. ^{1}H NMR and ^{13}C NMR were recorded in d_6DMSO at 300 and 75 MHz, respectively, on a Varian Gemini spectrometer, and are referenced to the solvent. Electrospray mass spectra were recorded on a PE Sciex API 100 benchtop system employing the Turbo IonSpray ion source. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ.

Dihydrophenolphthalein (1).- Phenolphthalein (5.0 g, 15.7 mmol) was dissolved in a solution of sodium hydroxide (1.29 g, 32.2 mmol) in water (50 mL), forming a dark purple solution, and zinc dust (20.0 g, 314 mmol) was added. The reaction mixture was heated to reflux with rapid stirring for 96 hrs; fresh zinc dust (3.0 g, 46 mmol) was added every 24 hrs, until a gray suspension (due to unreacted Zn dust) had formed. The suspension was filtered while hot and washed with water (~20 mL). The filtrate was acidified with 30% HOAc to pH 3, and the resulting white precipitate was collected and washed with ~100 mL water. The white solid was dried *in vacuo* to yield 4.991 g (99%) of **1** as a white solid.

^{1}H NMR: δ 9.24 (br, 2H), 7.69 (dd, 1H, J = 7.7 Hz, 1.2 Hz), 7.39 (t, 1H, J = 7.6 Hz), 7.25 (t, 1H, J = 7.5 Hz), 6.97 (d, 1H, J = 7.7 Hz), 6.77 (d, 4H, J = 8.6 Hz), 6.64 (d, 4H, J = 8.7 Hz), 6.40 (s, 1H); ^{13}C NMR: δ 155.4, 144.8, 134.4, 130.7, 130.0, 129.8, 125.8, 114.9, 49.5; ESMS: 319.2 $(M - H)^-$.

Anal. Calcd for $C_{20}H_{16}O_4 \cdot 0.33\ H_2O$: C, 73.62; H, 5.15. Found C, 73.57; H, 4.91.

Crystallization from aqueous MeOH provided an analytical sample, mp 232-235°, *lit*[9] 233-234°.

Representative Experimental Procedure. Dihydrophenolphthalein Amide (2a).- To a solution of dihydrophenolphthalein (100 mg, 0.31 mmol) in anhydrous DMF (300 µL) were added N-hydroxysuccinimide (53 mg, 0.46 mmol) and dicyclohexylcarbodiimide (71 mg, 0.34 mmol). After stirring for 8 hrs, the reaction mixture was diluted with dichloromethane (2 mL) and the insoluble solid filtered and washed with 1 mL dichloromethane. Ammonia in ethanol (2M, 2.0 mL, 4.0 mmol) was then added to the combined filtrates, and the mixture was stirred for 14 hr at ambient temperature and purified directly by preparative reversed phase HPLC, eluting with 30% CH_3CN/70% 0.05% aqueous CF_3COOH. Concentration of the combined fractions on a rotovap and lyophilization produced 77 mg (77%) of an amorphous white solid.

^{1}H NMR: δ 9.11 (br, 2H), 7.62 (s, 1H), 7.30 (m, 3H), 7.20 (t, 1H, J = 7.4 Hz), 6.98 (d, 1H, J = 7.7 Hz), 6.81 (d, 4H, J = 8.5 Hz), 6.64 (d, 4H, J = 8.5 Hz), 6.01 (s, 1H); ^{13}C NMR: δ 171.2, 155.4, 142.3, 137.4, 134.5, 129.9, 129.6, 128.8, 127.1, 125.6, 114.8, 49.3; ESMS: 318.2 $(M - H)^-$.

Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22; H, 5.37; N, 4.39. Found C, 75.36; H, 5.63; N, 4.10

Crystallization from aqueous MeOH provided an analytical sample, mp 236-238°.

Dihydrophenolphthalein N-Methylamide (2b).- 85% yield; ^{1}H NMR: δ 9.21 (s, 2H), 8.00 (m, 1H), 7.30 (m, 1H), 7.23 (m, 2H), 6.99 (d, 2H, J = 7.7 Hz), 6.82 (d, 4H, J = 8.5 Hz), 6.64 (d, 4H, J = 8.5 Hz), 5.86 (s, 1H), 2.60 (d, 3H, J = 4.4 Hz); ^{13}C NMR: δ 169.6, 155.4, 142.6, 137.7, 134.2, 129.9, 129.4, 128.8, 127.0, 125.6, 114.8, 49.6, 25.9; ESMS 322.2 $(M - H)^-$.

Anal. Calcd for $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found C, 75.48; H, 5.82; N, 4.18

Crystallization from aqueous MeOH provided an analytical sample, mp 243-245°.

Dihydrophenolphthalein N-(Carboxymethyl)amide (2c).- 67% yield; ^{1}H NMR: δ 9.32 (s, 2H), 8.55 (t, 1H, J = 5.7 Hz), 7.30 (m, 2H), 7.23 (t, 1H, J = 7.2 Hz), 7.09 (d, 2H, J = 7.5 Hz), 6.86 (d, 4H, J = 8.5 Hz), 6.62 (d, 4H, J = 8.5 Hz), 5.88 (s, 1H), 3.79 (d, 2H, J = 5.8 Hz); ^{13}C NMR: δ 171.2, 169.6, 155.4, 142.6, 137.0, 134.5, 129.9, 129.7, 129.1, 127.0, 125.6, 114.8, 49.4, 41.0; ESMS 376.2 $(M - H)^-$.

Anal. Calcd for $C_{22}H_{19}NO_5 \cdot 0.35\ CF_3COOH$: C, 65.34; H, 4.67; N, 3.36.

Found: C, 65.44; H, 4.35; N, 3.55

Crystallization from aqueous MeOH provided an analytical sample, mp 147-152° (dec).

Dihydrophthalein N-(2-Aminopropyl)amide trifluoroacetate (2d).- 90% yield; ^{1}H NMR: δ 8.30 (t, 1H, J = 5.7 Hz), 7.70 (br, 2H), 7.32 (m, 1H), 7.26 (m, 2H), 6.99 (d, 2H, J = 8.9 Hz), 6.81 (d, 4H, J = 8.2 Hz), 6.65 (d, 4H, J = 8.6 Hz), 5.87 (s, 1H), 3.17 (q, 2H, J = 6.3 Hz), 2.71 (m, 2H), 1.66 (m, 2H); ^{13}C NMR: δ 169.7, 155.5, 142.6, 137.2, 134.1, 129.9, 129.5, 129.1, 127.1, 125.7, 114.9, 49.6, 36.7, 35.8, 27.3; ESMS 377.3 $(M + H)^+$.

Anal. Calcd for $C_{25}H_{25}F_3N_2O_5 \cdot 0.10\ CF_3COOH$: C, 60.31; H, 5.04; N, 5.58.

Found: C, 60.16; H, 5.12; N, 5.89

Crystallization from $CHCl_3$/MeOH provided an analytical sample, mp 218-221°.

Dihydrophenolphthalein N-(4-Hydroxybutyl)amide (2e).- 71% yield; ^{1}H NMR: δ 9.20 (br, 2H), 8.10 (t, 1H, J = 5.5 Hz), 7.32-7.20 (m, 4H), 6.98 (d, 2H, J = 7.7 Hz), 6.80 (d, 4H, J = 8.3 Hz), 6.63 (d, 4H, J = 8.6 Hz), 5.88 (s, 1H), 3.80 (br, 1H), 3.36 (t, 2H, J = 6.1 Hz), 3.08 (m, 2H), 1.36 (m, 4H); ^{13}C NMR: δ 169.0, 155.4, 142.5, 137.8, 134.3, 129.9, 129.4, 128.7, 127.1, 125.6, 114.8, 60.4, 49.5, 36.7, 29.9, 25.6; ESMS: 390.5 $(M - H)^-$.

Anal. Calcd for $C_{24}H_{25}NO_4 \cdot 0.5\ H_2O$: C, 71.98; H, 6.54; N, 3.50. Found C, 71.96; H, 6.18; N, 3.43

Crystallization from aqueous MeOH provided an analytical sample, mp 176-178°.

Representative Experimental Procedure. Phenolphthalein Lactam (3a).- To a solution of amide **2a** (40 mg, 0.12 mmol) in methanol (2.0 mL) was added dichlorodicyanoquinone (DDQ, 86 mg, 0.36 mmol). The reaction was stirred for 14 hr at ambient temperature. Purification by preparative reversed phase HPLC, eluting with 31% CH_3CN/69% 0.05% aqueous CF_3COOH, provided 30 mg (75%) of **3a** as a colorless solid.

^{1}H NMR: δ 9.49 (s, 1H), 9.35 (br, 2H), 7.65 (d, 1H, J = 7.4 Hz), 7.53 (m, 3H), 7.00 (d, 4H, J = 8.8 Hz), 6.67 (d, 4H, J = 8.6 Hz); ^{13}C NMR: δ 168.2, 156.5, 150.9, 133.7, 131.8, 131.0, 128.1, 128.0, 124.6, 123.1, 114.9, 69.3; ESMS: 318.2 $(M + H)^+$.

Anal. Calcd for $C_{20}H_{15}NO_3 \cdot 0.30\ CF_3COOH$: C, 70.38; H, 4.39; N, 3.98.

Found: C, 70.26; H, 4.03; N, 4.36.

Crystallization from water provided an analytical sample, mp 270-272°.

Phenolphthalein N-Methyllactam (3b).- 85% yield; ^{1}H NMR: δ 9.56 (br 2H), 7.70 (d, 1H, J = 7.4 Hz), 7.54 (td, 1H, J = 7.4, 1.4 Hz), 7.45 (td, 1H, J = 7.1, 0.8 Hz), 7.36 (d, 1H, J = 7.7 Hz), 6.88 (d, 4H, J = 8.7 Hz), 6.72 (d, 4H, J = 8.8 Hz), 2.68 (s, 3H); ^{13}C NMR: δ 166.8, 157.0, 151.2, 132.0, 130.0, 129.9, 129.0, 128.0, 123.7, 122.8, 115.3, 73.9, 25.4; ESMS: 332.2 $(M + H)^+$.

Anal. Calcd for $C_{21}H_{17}NO_3$•0.25 CF_3COOH: C, 71.36; H, 5.36; N, 3.87.

Found: C, 71.14; H, 5.16; N, 4.27

Crystallization from aqueous MeOH provided an analytical sample, mp 234-236°.

Phenolphthalein N-(Carboxymethyl)lactam (3c).- 81% yield; ^{1}H NMR: δ 9.52 (br, 2H), 7.72 (d, 1H, J = 6.9 Hz), 7.57 (td, 1H, J = 7.4, 1.4 Hz), 7.47 (td, 1H, J = 7.4, 0.9 Hz), 7.40 (d, 1H, J = 7.7 Hz), 6.92 (d, 4H, J = 8.8 Hz), 6.67 (d, 4H, J = 8.6 Hz), 3.98 (s, 2H); ^{13}C NMR: δ 168.6, 167.1, 157.0, 151.3, 132.4, 130.4, 129.7, 128.1, 123.9, 122.9, 115.1, 74.3, 41.8; ESMS: 376.2 $(M + H)^+$.

Anal. Calcd for $C_{22}H_{17}NO_5$: C, 70.39; H, 4.56; N, 3.73. Found: C, 70.43; H, 4.90; N, 3.95

Crystallization from aqueous MeOH provided an analytical sample, mp 256-260° (dec).

Phenolphthalein N-(Aminopropyl)lactam trifluoroacetate (3d).- 86% yield; ^{1}H NMR: δ 9.35 (br 2H), 7.70 (d, 1H, J = 7.4 Hz), 7.56 (t, 1H, J = 7.4 Hz), 7.46 (t, 1H, J = 7.4 Hz), 7.36 (d, 1H, J = 7.4 Hz), 6.93 (d, 4H, J = 8.7 Hz), 6.93 (d, 4H, J = 8.8 Hz), 3.48 (m, 6H), 1.13 (m, 2H); ^{13}C NMR: δ 167.6, 156.5, 157.2, 151.5, 132.4, 130.4, 129.7, 128.9, 128.1, 123.8, 122.8, 115.4, 74.6, 37.9, 36.7, 25.8; ESMS: 375.2 $(M + H)^+$.

Anal. Calcd for $C_{25}H_{23}F_3N_2O_5$•0.65 CF_3COOH: C, 60.11; H, 4.70; N, 5.77.

Found: C, 59.97; H, 4.44; N, 6.23

Crystallization from $CHCl_3$/MeOH provided an analytical sample, mp 231-233°.

Phenolphthalein N-(4-Hydroxybutyl)lactam (3e).- 80% yield; ^{1}H NMR: δ 9.55 (br 2H), 7.67 (d, 1H, J = 6.8 Hz), 7.52 (t, 1H, J = 7.4 Hz), 7.43 (t, 1H, J = 7.4 Hz), 7.37 (d, 1H, J = 7.4 Hz), 6.93 (d, 4H, J = 8.8 Hz), 6.71 (d, 4H, J = 8.5 Hz), 3.70 (t, 2H, J = 7.6 Hz), 3.10 (t, 2H, J = 6.6 Hz), 1.12 (m, 2H), 0.78 (m, 2H); ^{13}C NMR: δ 166.9, 157.0, 151.5, 132.0, 130.8, 128.9, 127.9, 123.6, 122.6, 115.3, 74.3, 60.5, 30.1, 24.2; ESMS: 390.6 $(M + H)^+$.

Anal. Calcd for $C_{24}H_{23}NO_4$•0.30 CF_3COOH: C, 69.74; H, 5.54; N, 3.31.

Found: C, 69.42; H, 5.26; N, 3.52

Crystallization from aqueous MeOH provided an analytical sample, mp 226-228°.

REFERENCES

1. D. Wild, *The Immunoassay Handbook.* Stockton Press, New York, 1994. F. H. Kasten, Chapter 2. In: *Fluorescent and Luminescent Probes for Biological Activity*, W. T. Mason, Ed.; Wiley Interscience, New York, 1983.

2. *Handbook of Fluorescent Probes and Research Chemicals*; 6th ed.; R. P. Haugland, Ed.; Molecular Probes: Eugene, OR, 1996, p. 22-32.

3. W. R. Orndorff and A. J. Hemmer, *J. Am. Chem. Soc.*, **49**, 1272 (1949).

4. U. Anthoni, C. Christophersen, P. Nielsen, A. Puschl and K. Schaumburg, *Structural Chem.*, **3**, 161 (1995).

5. R. Marekuszewski and H. Diehl, *Talanta*, **27**, 937 (1980).

6. M. Adamczyk and J. Grote, *Tetrahedron Lett.*, **41**, 807 (2000).

7. M. Adamczyk and J. Grote, *Bioorg. Med. Chem. Lett.*, **10**, 1539 (2000).

8. Merck Index, Tenth Edition, p. 347.

9. M. Blicke and O. Patelski, *J. Am. Chem. Soc.*, **58**, 276 (1936).

10. The data for product obtained by DDQ oxidation of phenolphthalein hydroxyamide are: ESMS (M + H)+ at 334.2; ^{1}H NMR: δ 11.2 (s, 1H), 9.62 (br, 2H), 7.88 (dd, 1H, J = 7.3, J = 1.8 Hz), 7.51 (m, 3H), 6.83 (d, 4H, J = 8.6 Hz), 6.72 (d, 4H, J = 8.8 Hz), 6.65 (d, 1H J = 7.1 Hz); ^{13}C NMR: δ 162.8, 157.3, 143.4, 132.1, 131.4, 129.8, 128.0, 127.0, 126.4, 126.1, 114.6, 88.1. Although the mass spectrum may be consistent for either the five-membered hydroxyspirolactam or a six-membered oxolactam, the ^{13}C NMR differs from other spirolactams (N-C9 at ~74.0 ppm), in that it shows a C9 resonance at 88.1 ppm, indicative of a C9 substituted with oxygen. In contrast, the N-oxo and N- amino fluorescein spirolactams (reference 6 above) display resonances similar to other N-alkyl species (all show C9 ~64 ppm).

****

1,3-DIPOLAR CYCLOADDITIONS OF 3-PHENYLSYDNONE WITH ACETYLENE DICARBOXYLATES IN SUPERCRITICAL CARBON DIOXIDE

Submitted by A. E. McGowin*, L. Jackson, L. W. Marshall and K. Turnbull
(08/04/00)

Department of Chemistry
Wright State University, Dayton, OH 45435

Sydnones undergo 1,3-dipolar cycloadditions with alkynes or alkenes to form pyrazoles or related species.[1] This process is of considerable interest and has been most successful in solvents such as toluene or xylene at temperatures ranging from 110-150° with alkynes bearing electron-withdrawing groups.