179287

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Project Title: New Reagents for the Development of Latent Fingerprints

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Project contact person: Dr. Richard Rau

Contact person's telephone number: (202) 307-6394

Grantee name and address: Professor Madeleine M. Joullié

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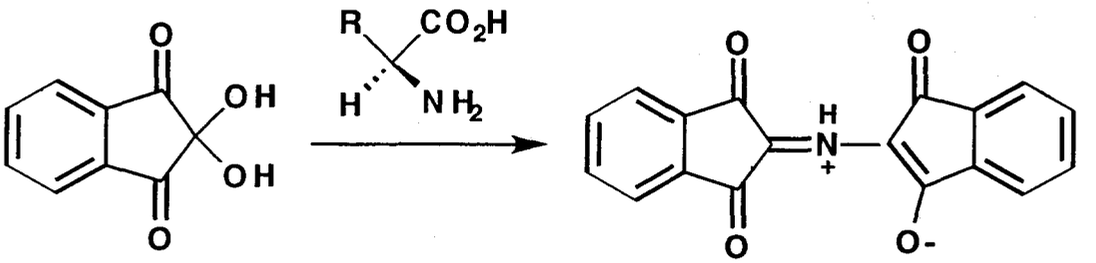
Project description:

Ia. The presentation of physical evidence is the core of any case in our criminal justice system. Irrefutable evidence which can establish the identity of the perpetrator is the prosecution's goal. DNA fingerprinting is currently at the forefront of many criminal cases; however, there is still debate, even among forensic experts, regarding the handling of samples, population matches, and reproducibility of results. In many instances, no DNA samples can be recovered from a crime scene, or the samples are old or damaged. DNA fingerprinting is also a costly and time-consuming process, and is often unavailable to the average law enforcement officer working on a full workload of cases. In contrast, fingerprints are known to be unique to each individual and a definite number of "matches" between a print obtained as evidence and one on record have been established as acceptable. The basics of obtaining usable prints are standard among forensic scientists and law enforcement officers. The computerized collection of prints of known criminals which is on-line will drastically decrease the time spent searching for a match, and will allow for searches against a broader population. One of the most affordable and useful reagents for visualization of latent fingerprints on porous surfaces such as paper, wood, and

walls is ninhydrin (2,2-dihydroxy-1,3-indanedione). Ninhydrin was first made in 1910 by the English chemist Siegfried Ruhemann,[1] who also investigated its reaction with amines and amino acids to form a colored compound known as Ruhemann's Purple (RP).



Ninhydrin
or 2,2-dihydroxy-1,3-indanedione

Ruhemann's Purple (RP)

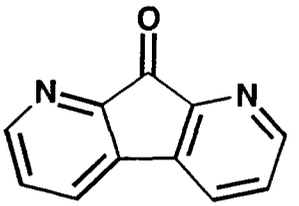
The significance of this discovery to the development of latent fingerprints went unnoticed until 1954 when Odén and von Hoffsten reported the use of ninhydrin as a fingerprint development reagent.[2] The reaction between ninhydrin and the amino acids secreted from the ecrine glands form Ruhemann's purple (RP).[3] Although the relative amount of amino acids in a fingerprint residue is low compared to the amount of salts and fatty acids, they form a unique, lasting picture of an individual.

Ib. In 1991, Almog reported that ninhydrin and modified ninhydrins were the best reagents for use on paper.[4] Therefore, it was our goal to enhance the properties of ninhydrin via structural modifications to create a superior print development reagent which would be useful to professionals involved in identification of latent fingerprints. We proposed to synthesize different ninhydrin analogs to be tested for latent prints visualization.

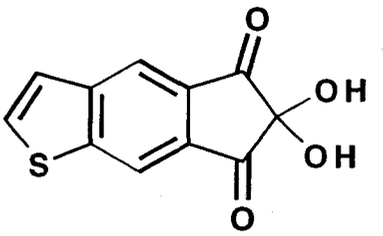
IIa. A variety of fingerprint-developing reagents have been reported. For fresh prints, fuming superglue fixes prints on non-porous surfaces such as

metals. Dusting with a fine powder also aids in visualization of fresh prints. However, criminals rarely leave their mark cleanly and on surfaces which are conveniently treated to afford clear prints. Evidence may also not be uncovered until after the prints have aged significantly. In 1990, Pounds and co-workers introduced the reagent 1,8-diazafluorenone (DFO), which is commercially available and used in the United Kingdom.[5,6] Unlike ninhydrin, which reacts with the amino acids in a fingerprint residue to produce a violet-colored print, DFO gives only a weakly colored red print. The main feature of this reagent is its ability to give a fluorescent print with little background interference. This "glow" can be seen when the fingerprint is illuminated with a light of certain wavelength. Ninhydrin itself exhibits a weaker fluorescence when the originally developed print is treated with a metal salt solution such as zinc chloride, but has the advantage of providing an initial print that is highly colored.

In a 1993 report by Cantu et. al., one reagent previously prepared by our group, thieno[f]ninhydrin, was reported to provide a print which was initially as highly colored as ninhydrin, but after treatment with a zinc chloride solution, it gave a print which exhibited stronger fluorescence than DFO.[7] When the print is initially developed with a solution of the reagent containing acetic acid, a fluorescent complex was observed without the use of a metal for secondary development. Thus, we had succeeded in preparing a reagent which combined the most desirable properties of reagents known at that time. However, since the compound was difficult to make, and would be too expensive to produce on a commercial scale, we had to design a reagent which would possess the properties of a superior reagent but would be amenable to a cost-effective, large-scale production.



1,8-Diazafluorenone (DFO)

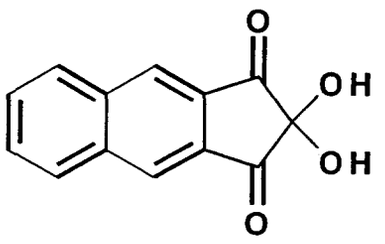


Thieno[f]ninhydrin

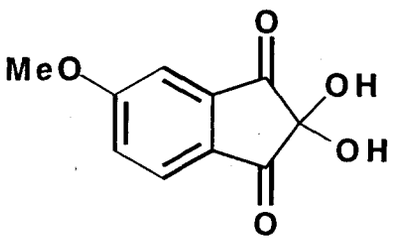
IIb. We next created a list of desirable properties of a superior reagent. There must be a rapid rate of reaction with the amino acids in the fingerprint, which would produce a colored print. The developed print must be fluorescent, preferably without secondary treatment with a metal salt solution. The maximum fluorescence emission should occur at a wavelength greater than 550 nanometers to avoid competition with optical brighteners in paper, and to match the visual acuity of the human eye. The fluorescence should also exhibit a high quantum efficiency. The reagent must be soluble in solvents that do not smear the print, and fluorinated solvents which were previously used are now banned. The compound must be stable on storage for long periods of time. The reagent must be non toxic and safe to handle. It should be economical, and its synthesis suited to large-scale preparations. Unfortunately, one cannot predict *a priori* which reagent will possess superior properties. Therefore, we proposed to synthesize a variety of reagents and have them evaluated by forensic scientists at the United States Secret Service and other law enforcement agencies.

IIc. Until recently, protocols available for the synthesis of ninhydrin analogs were inefficient. Our group reported novel approaches to the synthesis of benzo[f]ninhydrin, 5-methoxyninhydrin, 5-(methylthio)ninhydrin, and thieno[f]ninhydrin. We then synthesized 5-phenylninhydrin in which the conjugation of a phenyl group with the ninhydrin nucleus was expected to result in an absorption at longer wavelengths. However, this reagent gave

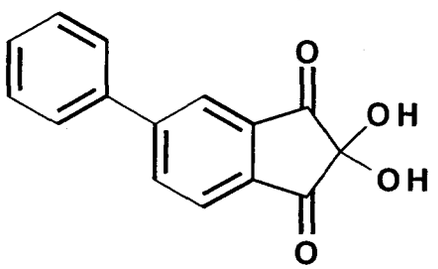
disappointing results when compared with the previous compounds. Since it is known that replacement of a phenyl ring by a thiophene ring in some triarylmethane dyes such as Malachite Green produces a marked bathochromic shift, we set out to prepare the 5-(2-thienyl)ninhydrin (2-THIN), which has proven to be a superior reagent. However, based on our experience with reagents which were designed to be superior and showed properties only equal to ninhydrin itself, we are still unable to predict which reagents will possess the properties required for a superior reagent.



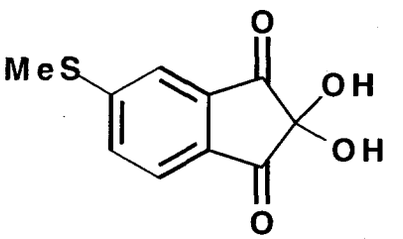
Benzo[f]ninhydrin



5-Methoxyninhydrin



5-Phenylninhydrin



5-(Methylthio)ninhydrin

IIIa,b. The methodology developed for the synthesis of the aryl-substituted ninhydrin analogs, most notably "2-THIN", is shown in **Scheme 1**. A palladium-catalyzed cross-coupling reaction of 5-bromo-ninhydrin dimethyl acetal and an aryl boronic acid, aryl tributylstannane, or an electron-sufficient aryl group is the key to these syntheses. We investigated several routes to a common coupling partner (5-bromoninhydrin dimethyl acetal), and are now able to prepare this compound on a large scale that is commercially viable

(**Scheme 2**). Preparation of the 2-thienyl boronic acid and the 2,5-bis(tributylstannyl)-thiophene are shown in **Scheme 3**. The "2-THIN" reagent can also be prepared by the reaction of thiophene with the bromoacetal, in an improved yield.[8] The synthesis of "2-THIN" was performed on a 100 gram scale at Vinfer Ltd. in Belfast, Ireland last year. The company is interested in the commercial production of this superior reagent. A list of compounds prepared by the new methods we developed is shown in **Table 1**.

One of the synthetic steps responsible for a decreased yield is the last step, the deprotection of the dimethyl acetal to afford the free ninhydrin. Much time and effort has been spent on improving this reaction, but even with common and exotic deprotection protocols known to organic chemists, side reactions which give undesired products are still a problem.

We have designed and synthesized several superior reagents which contain dual reactive sites (**Schemes 4 and 5**) The resulting polymeric Ruhemann's purple formed would be expected to extend the conjugation of the system, and increase the fluorescence of the developed print. Unfortunately, many of these "Janus" ninhydrins had poor solubility in acceptable solvents for fingerprint development, and thus showed poor performance as reagents.

The ban on the very useful chlorofluorocarbons such as trichlorotrifluoroethane has greatly disrupted police work.[9,10] Therefore, a search for acceptable substitute solvents has been initiated but so far it has not proven fruitful. We have found that some derivatives of ninhydrin, resulting from its reaction with higher molecular weight alcohols (hemiacetals), undergo the same color-forming reactions and are more soluble in organic solvents such as ethyl acetate, methylene chloride, or toluene than ninhydrin (**Table II**). This approach affords a solution to the ban on chlorofluorocarbons.

IVa. To summarize, several synthetic approaches were developed and evaluated to provide a total of 21 new substituted ninhydrins. These reagents were evaluated for fingerprint visualization by forensic experts at the US Secret Service and other law enforcement agencies both in this country, England, Switzerland, Australia, and Israel. The new methodology developed afforded two complimentary reagents, which displayed similar fingerprint developing properties (2-THIN and 3-THIN). 2-THIN was produced on a large scale by Vinfer Ltd. in North Ireland to investigate its potential for commercialization.[8]

Finally, because of the importance of formulations in the development of new fingerprint visualization reagents and as a potential solution to the problems caused by the ban on chlorofluorocarbons, we developed structural modifications of ninhydrin-based reagents to expand their solubilities in suitable organic solvents (methylene chloride, toluene, hexanes etc.).

IVb. Our research has resulted in improved reagents for latent fingerprint development. Additionally, it has provided a suitable alternative to the technical problems created by the ban on chlorofluorocarbons. Our results are useful to all law enforcement agencies both in this country and abroad.

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(4) Almog, J.; Hirshfeld, A.; Frank, A.; Sterling, J.; Leonov, D. *J. Forensic Sci.* **1991**, *36*, 104.

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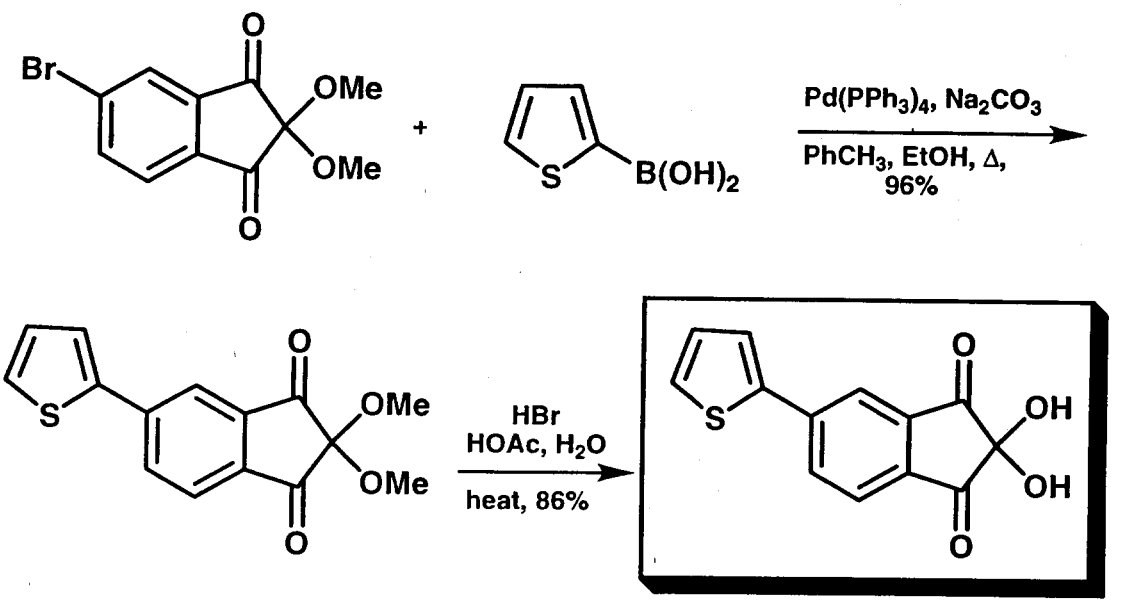
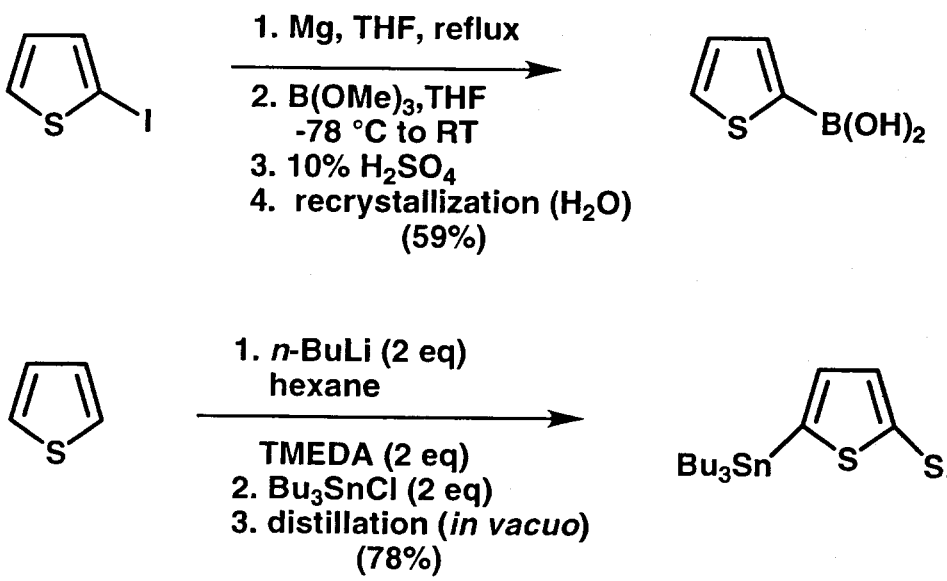
(10) Zurer, P. *Chem. Eng. News* **1995**, 9.

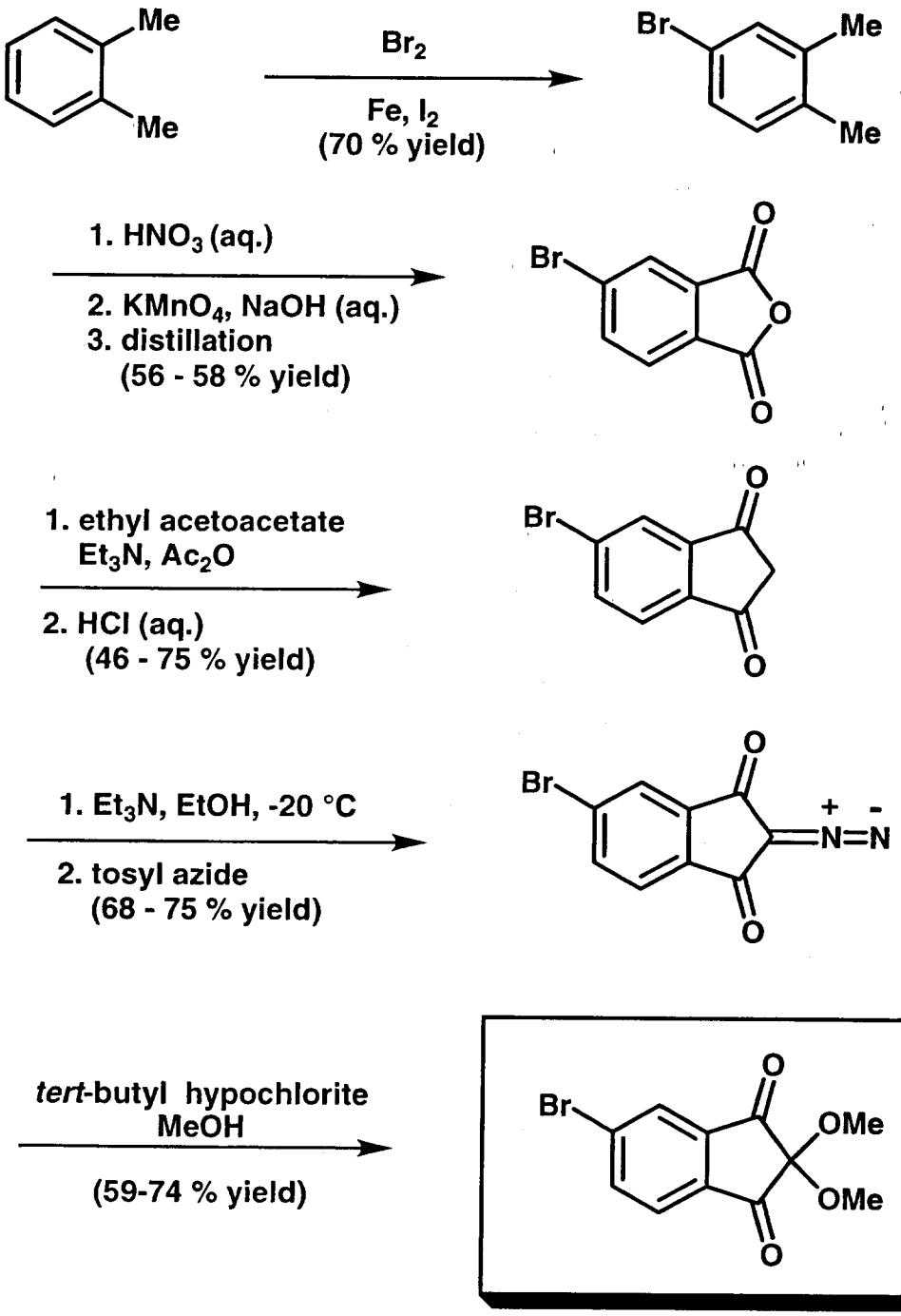
Scheme 1



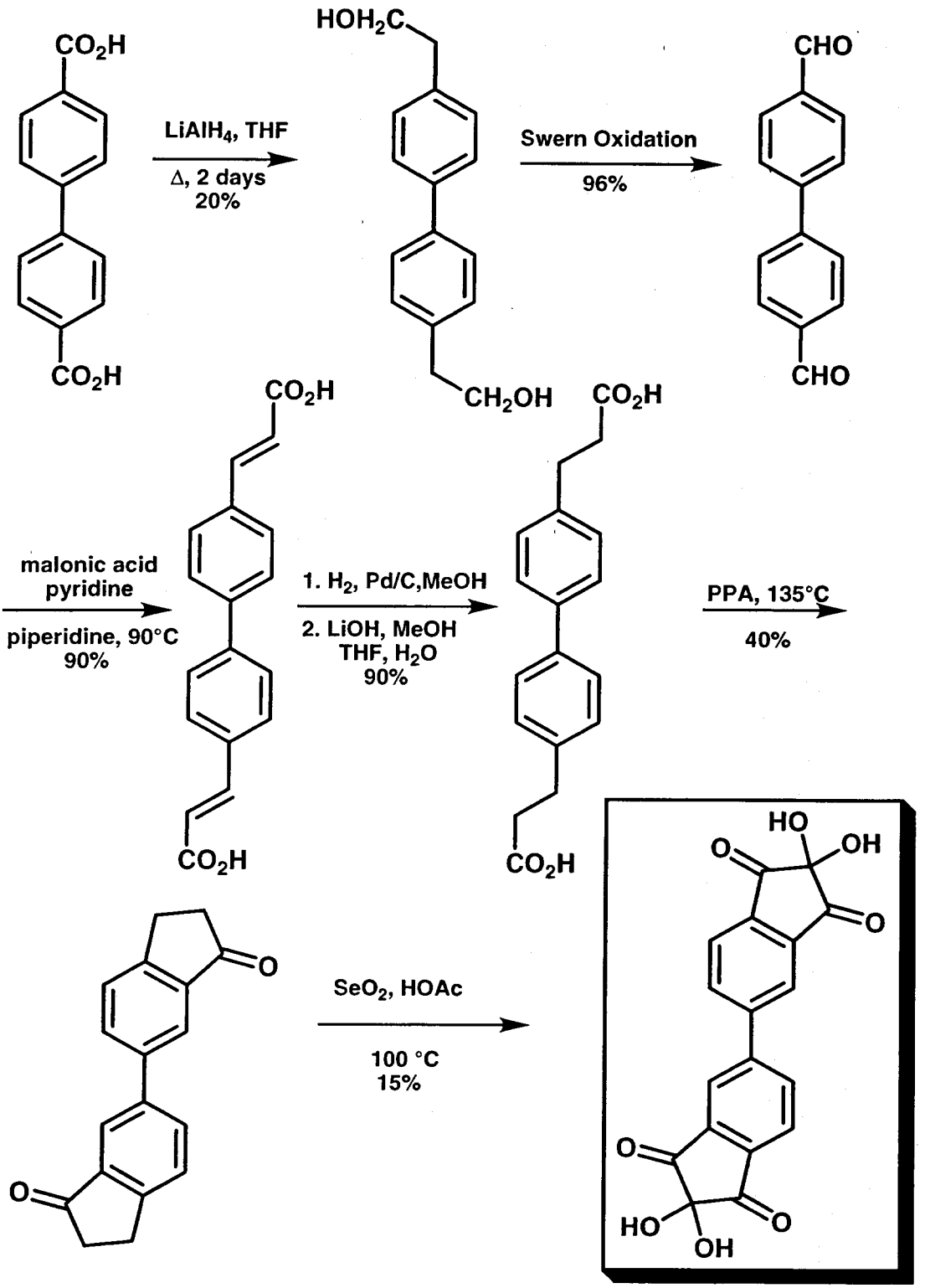
Scheme 3



Scheme 2



Scheme 4



Scheme 5

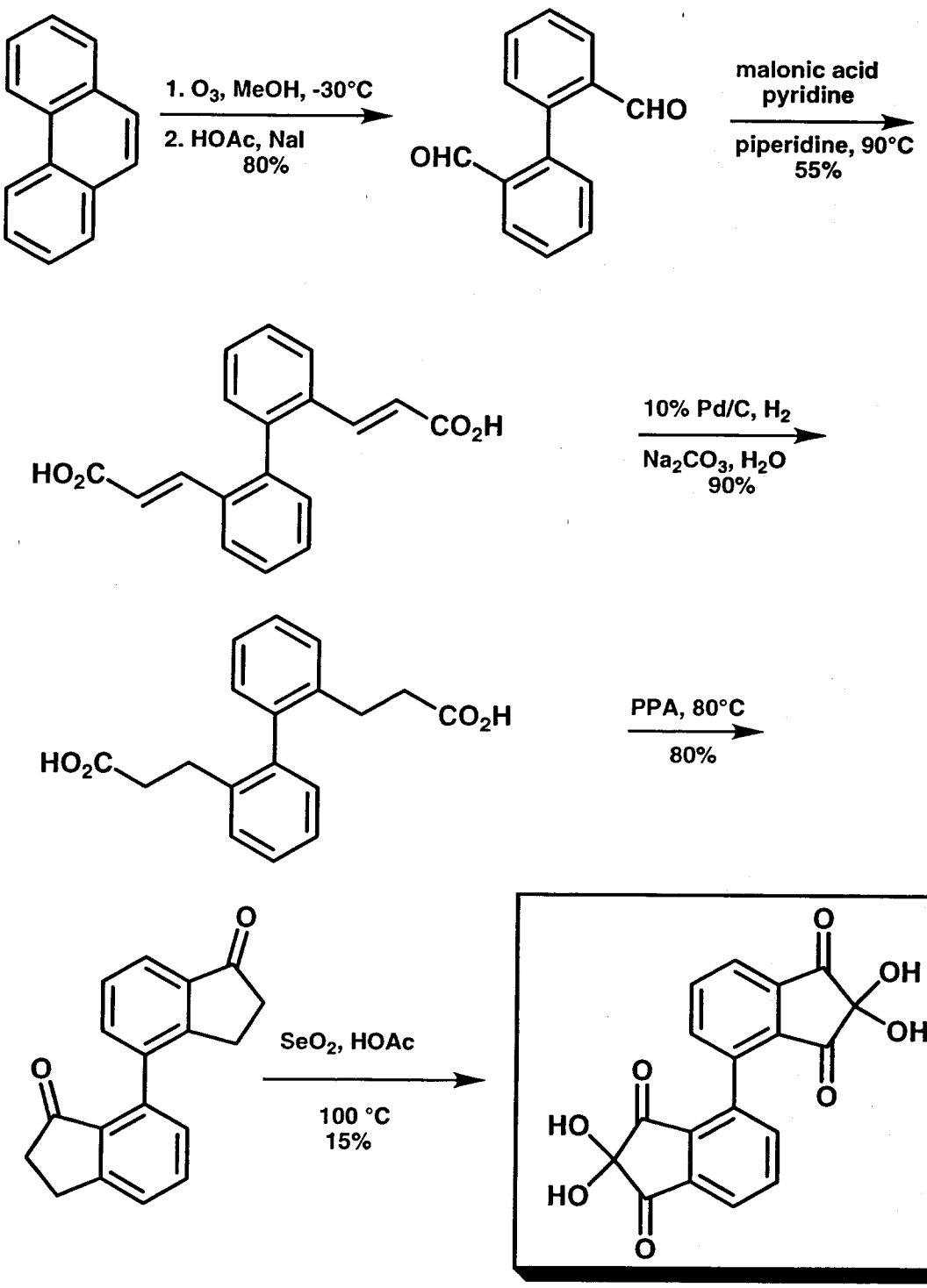
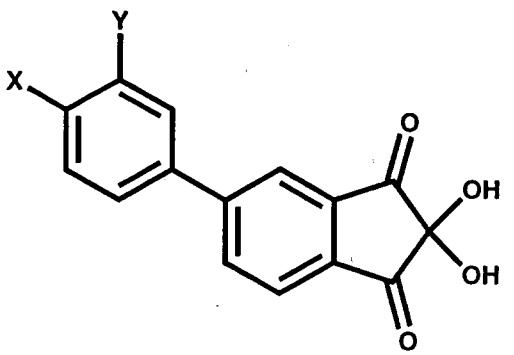
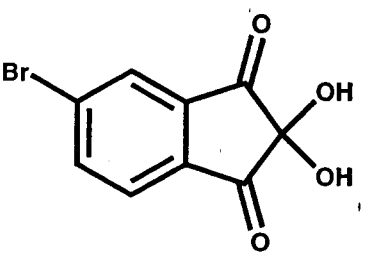


Table 1
New ninhydrin analogs



	X =	Y =
2	CH_3S	H
3	Ph	H
4	CH_3O	H
5	HO	H
9	$(CH_3)_2N$	H
10	H	H
11	$-OCH_2O-$	

1



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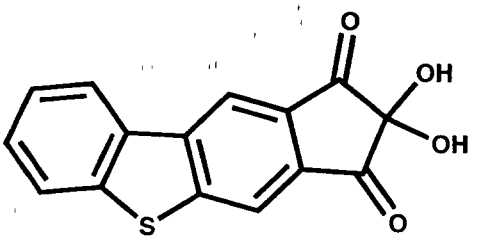

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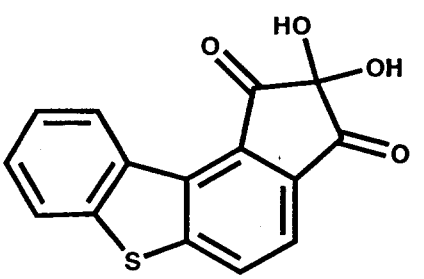

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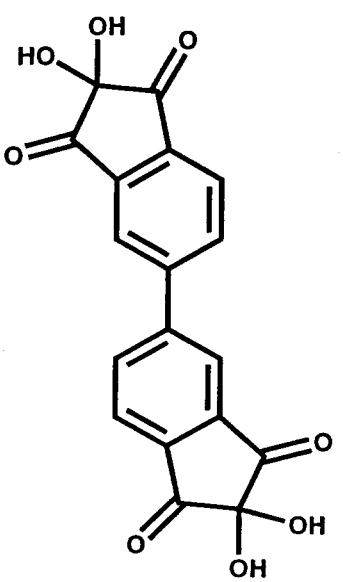

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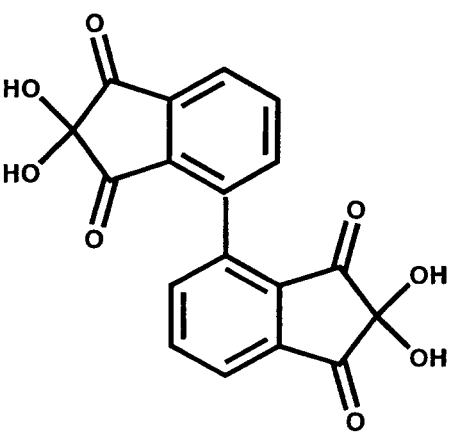
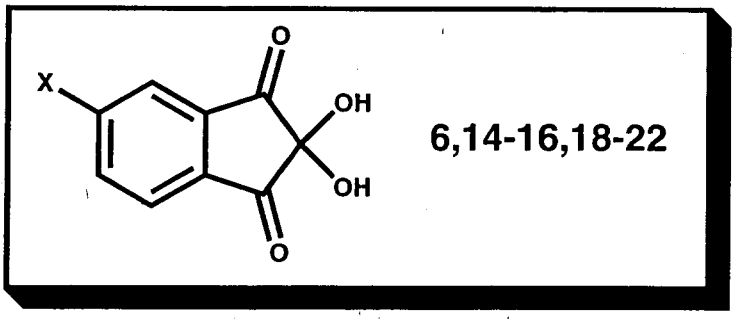


Table I

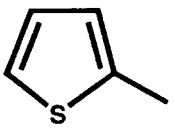
New ninhydrin analogs (continued)



6,14-16,18-22

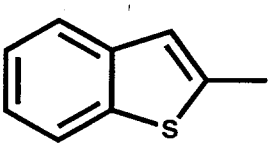
X =

6



2-THIN

14



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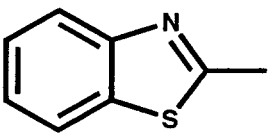

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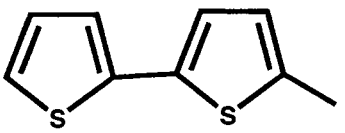

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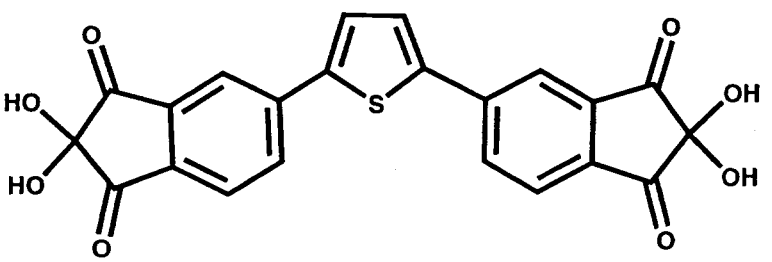
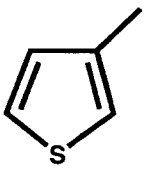


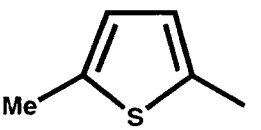
X =

18



3-THIN

19



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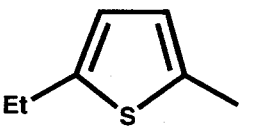

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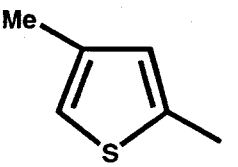

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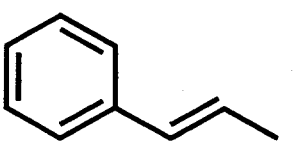
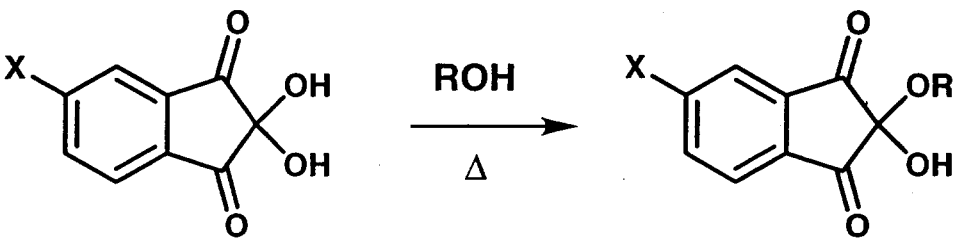


Table II
Hemiacetals



17 - 23

	X	R
17	H	Me
18	H	Et
19	H	*n*-Pr
20	H	*i*-Pr
21	H	*n*-Bu
22	H	*i*-Am
23	2-(5-ethyl-)thienyl-	Et

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Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323

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combined the most desirable properties of reagents known at that time. However, since the compound was difficult to make, and would be too expensive to produce on a commercial scale, we had to design a reagent which would possess the properties of a superior reagent but would be amenable to a cost-effective, large-scale production.

Until recently, protocols available for the synthesis of ninhydrin analogs were inefficient. Our group reported novel approaches to the synthesis of benzo[f]ninhydrin, 5-methoxyninhydrin, 5-(methylthio)ninhydrin, and thieno[f]ninhydrin. We then synthesized 5-(2-thienyl)ninhydrin (2-THIN), which has proven to be a superior reagent.

The ban on the very useful chlorofluorocarbons such as trichlorotrifluoroethane has greatly disrupted police work.[9,10] Therefore, a search for acceptable substitute solvents has been initiated but so far it has not proven fruitful. We have found that some derivatives of ninhydrin, resulting from its reaction with higher molecular weight alcohols (hemiacetals), undergo the same color-forming reactions and are more soluble in organic solvents such as ethyl acetate, methylene chloride, or toluene than ninhydrin. This approach affords a solution to the ban on chlorofluorocarbons.

To summarize, several synthetic approaches were developed and evaluated to provide a total of 21 new substituted ninhydrins. These reagents were evaluated for fingerprint visualization by forensic experts at the US Secret Service and other law enforcement agencies both in this country, England, Switzerland, Australia, and Israel. The novel methodology developed afforded two complimentary reagents, which displayed similar fingerprint developing properties (2-THIN and 3-THIN). 2-THIN was produced on a large scale by Vinfer Ltd. in North Ireland to investigate its potential for commercialization.[8]

Finally, because of the importance of formulations in the development of new fingerprint visualization reagents and as a potential solution to the problems caused by the ban on chlorofluorocarbons, we developed structural modifications of ninhydrin-based

reagents to expand their solubilities in suitable organic solvents (methylene chloride, toluene, hexanes etc.).

Our research has resulted in improved reagents for latent fingerprint development. Additionally, it has provided a suitable alternative to the technical problems created by the ban on chlorofluorocarbons. Our results are useful to all law enforcement agencies both in this country and abroad.

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