Modified method of synthesis of N-substituted dithioesters of amino acids and peptides in the Pinner reaction[1]

Witold Neugebauer, Eric Pinet, Munsok Kim, and Paul R. Carey

Abstract: An improved method for the synthesis of dithioesters of amino acids and peptides has been developed. The syntheses have been carried out from the nitriles. The addition of thiol to the nitrile derivative in the Pinner step of dithioester synthesis was activated with hydrogen fluoride. A few examples of dithioester synthesis using liquid HF are described. Some novel dithioesters, which are model compounds for resonance Raman spectroscopic studies of dithioacylpapain intermediates, are described.

Key words: dithioesters, amino acids, Pinner reaction, HF, isotopes.

Résumé : Une méthode améliorée de synthèse de dithioesters d'acides amines et de peptides a été développée. Les synthèses ont été accomplies à partir des nitriles correspondants. L'addition du thiol au dérivé nitrile lors de la synthèse du dithioester, pendant la reaction de Pinner, a été activée par le fluorure d'hydrogène. Nous décrivons plusieurs exemples de synthèse de dithioesters employant du HF liquide, ainsi que plusieurs nouveaux dithioesters,composé-modéles employés dans les études des formes passagères de la dithioacylpapaine par la spectrométrie de résonance de Raman.

Mots clés : dithioesters, acides aminés, réaction de Pinner, HF, isotopes.

Introduction

Dithiocarboxylic acids and esters have recently attracted attention because of their structural and spectroscopic properties (1–5), and their application in organic synthesis (6). Since we have had difficulty in using available procedures for synthesis of dithioesters of N-substituted amino acids or small peptides, modification of the most general procedure seemed to be the best choice. Several methods of synthesis of this class of organo-sulphur compounds have been published (1, 7, 8) and reviewed (9). The most common route for dithioester synthesis is the procedure based on the Pinner reaction (10, 11). Thiols react with nitriles in the presence of hydrogen chloride to give imidothioesters and these are the key intermediates in the synthesis of dithioesters. In the modification reported here, the first step of the synthesis is dissolution of the nitrile in an excess of liquid hydrogen fluoride at a temperature below 0°C to most likely form the $[RCNH]^+HF_2^-$ adduct (eq. [1]) (12). In the presence of the third reaction component, mercaptan, thioimidate hydrofluoride is produced, $[RC(=NH_2)SR']^+HF_2^-$ (eq. [2]). Unlike the classic Pinner reaction with HCl saturated methylene chloride, all nitrile derivatives are completely soluble in the reaction mixture. Excess of hydrogen fluoride and mercaptan are removed under vacuum and the thioimidate hydrofluoride precipitated. It is then used under anhydrous conditions to react with H_2S in dry pyridine, forming the dithioester, eq. [3] (8).

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Witold Neugebauer,[2] Eric Pinet, Munsok Kim, and Paul R. Carey. Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, K1A 0R6, Canada.

[1] NRCC No. 39511.

[2] Author to whom correspondence may be addressed. Present address: Département de Pharmacologie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, QC, J1H 5N4, Canada. Telephone: (819) 820-6820. Fax: (819) 564 5400. E-mail: wneugeba@courrier.usherb.ca.

Experimental section

Materials

All solvents used (acetone, chloroform, toluene, diethyl ether, methanol) were of ACS purity and were purchased from BDH. Pyridine was purchased from BDH and distilled over KOH pellets. Ammonium chloride, *p*-halogen benzoyl chlorides, *p*-anisoyl chloride, and ethane thiol were purchased from Aldrich. Sodium cyanide, formaldehyde solution, and acetic acid were purchased from Anachemia Chemicals. Gaseous HF and H_2S were purchased from Matheson Gas, Canada. Anhydrous gas HF operations were undertaken in an HF apparatus (Biosearch). All final products were purified by crystallization and column chromatography on silica gel 60 (E. Merck) with toluene–chloroform—methanol as an eluent on medium pressure glass columns (Michel-Miller) 300 mm, Aldrich. Chromatographic purity of products was checked on TLC (silica gel, aluminium plates, E. Merck) in solvent systems A, chloroform–methanol 4:1, or B, chloroform. The final identity of the synthesized compounds was checked by FAB-MS spectrometry on Jeol, JMS-AX505H with dithiothreitol–dithioerythiol (DTT–DTE) or nitrobenzyl aldehyde (NBA) matrix, and in some cases by NMR spectroscopy.

Synthesis

General procedure of the Pinner reaction (2, 3, 3a, 3b, 3c, 3d, 3e, 3a1, 3a2, 3a3, 3a4, 3a5)

HF gas was liquified at the temperature of liquid nitrogen. N-substituted amino nitrile (10 mmol) and ethanethiol (13 mmol)

[1] $RCN + 2\,HF \longrightarrow [RCNH]^+ HF_2^-$

[2] $[RCNH]^+HF_2^- + R'SH \longrightarrow RC(=NH_2^+)-SR'\ HF_2^-$

[3] $RC(=NH_2^+)-SR'\ HF_2^- + H_2S + \text{pyridine-N} \longrightarrow RC(=S)-SR' + \text{pyridine-N}\cdot HF + NH_4F$

[4] $NH_4Cl + HCH(=O) + NaCN \longrightarrow CH_2{=}NCH_2CN + NaCl + H_2O$

[5] $CH_2{=}NCH_2CN + 2\,C_2H_5OH + H_2SO_4 \longrightarrow H_2NCH_2CN{\cdot}H_2SO_4 + CH_2(OC_2H_5)_2$

[6] $H_2NCH_2CN{\cdot}H_2SO_4 + X\text{-C}_6H_4\text{-C(=O)-Cl} + 2\,NaOH \longrightarrow$

$X\text{-C}_6H_4\text{-C(=O)-}NHCH_2CN + Na_2SO_4 + H_2O$

X= H, F, Cl, Br, I, OCH_3

were placed in a polyethylene reactor with a stirrer, at liquid nitrogen temperature, while HF (3 mL) was added. The reaction vessel was placed in the Biosearch HF apparatus and the reaction was held at 0°C and stirred for 1 h. The excess HF and ethanethiol were then evaporated at room temperature under a water aspirator and finally with an oil pump. The thioimidoester salts produced were precipitated (or solidified) by the addition of anhydrous diethyl ether. They were then decanted from ether, washed, decanted again, and used in the next synthetic step.

Dithiomethyl esters **4** and **6** require the use of liquified CH_3SH (instead of ethanethiol) prepared the same way as liquid HF. Similarly, thiomethyl ester (**1**) requires dry methanol. Dithioesters of dipeptides **5**, **6**, and **7** were formed after coupling of *N*-acetyl or *N*-methoxy phenylalanine to amino acetonitrile with DCC–pentafluorophenol in the standard manner (13).

Dithioesters

Pyridine , dried over KOH pellets, was saturated with dry H_2S at 0°C and thioimido esters were added while stirring and passing dry H_2S for 45 min. $H_2{}^{34}S$ generated from heating ^{34}S in paraffin was used for the isotopically substituted derivative (*3a*5). The coloured solutions (from light yellow to deep orange) were then poured into sufficient amounts of ice-cold 4 N HCl to neutralize the pyridine. The dithioesters were crystallized or precipitated and were collected by filtration and washed with cold water. The precipitates (crystals) were precipitated with water from acetone solution, then dissolved in chloroform and chloroform solution that was dried by rotary evaporation. The crude dithioesters were dissolved in a minimum volume of chloroform (1–3 mL) and purified on a silica gel column that was washed with toluene and solvent exchange under medium pressure from 10% chloroform in toluene (30 min) to 50% and 100% chloroform (65 min each). Finally the column was washed with methanol and toluene for the next purification. TLC identifications in solvent systems A and B were made, and the dithioesters were collected. Their identity was verified by FAB/MS spectrometry using NBA or DTT–DTE as a matrix.

General procedure for Strecker reaction (3a1, 3a2, 3a3, 3a4)

Isotopic substitutions were made using labelled substrates in a Strecker type synthesis; $Na^{13}CN$ (*3a*1), $H^{13}C(=O)H$ (*3a*2), $^{15}NH_4Cl$ (*3a*3), $DC(=O)D$ in D_2O (*3a*4).

NH_4Cl (2.16 g, 0.04) mol was dissolved in 6 mL (0.04 mol) formaldehyde at the ice-bath temperature and, while stirring, 2 g (0.04 mol) NaCN in 6 mL water was added dropwise over 2.5 h. Stirring continued for another 1.5 h and 1.33 mL of acetic acid was added dropwise over 1 h. After 2.5 h, the white precipitate was filtered, washed with cold water, and dried under high vacuum overnight. Yield 1.49 g (59.7%), mp 124–126°C, Schiff base derivative reaction [4].

CH_2NCH_2CN powder (1.49 g, 0.02 mol) was added to 1.3

Table 1. Products obtained in the Pinner reaction with liquid HF.

	Product	M + 1 (FAB/MS)	Yield (%)	Melting point (°C)
1	$CH_3C(=O)NHCH_2C(=S)OCH_3$	148	30	Oil
2	$CH_3C(=O)NHCH_2C(=S)SCH_2CH_3$	178	42	71
3	$C_6H_5C(=O)NHCH_2C(=S)SCH_2CH_3$	240	87	98
3*a*	p-$ClC_6H_4C(=O)NHCH_2C(=S)SCH_2CH_3$	274	67.8	94
3*b*	p-$BrC_6H_4C(=O)NHCH_2C(=S)SCH_2CH_3$	319	47.8	117
3*c*	p-$FC_6H_4C(=O)NHCH_2C(=S)SCH_2CH_3$	258	67.7	104
3*d*	p-$IC_6H_4C(=O)NHCH_2C(=S)SCH_2CH_3$	365	48.4	113
3*e*	p-$CH_3OC_6H_4C(=O)NHCH_2C(=S)SCH_2CH_3$	270	51.2	116
3*a*1	p-$ClC_6H_4C(=O)NHCH_2{}^{13}C(=S)SCH_2CH_3$	275	65.5	95
3*a*2	p-$ClC_6H_4C(=O)NH{}^{13}CH_2C(=S)SCH_2CH_3$	275	66.7	94
3*a*3	p-$ClC_6H_4C(=O){}^{15}NHCH_2C(=S)SCH_2CH_3$	275	40.2	95
3*a*4	p-$ClC_6H_4C(=O)NHCD_2C(=S)SCH_2CH_3$	276	61	99
3*a*5	p-$ClC_6H_4C(=O)NHCH_2C(={}^{34}S)SCH_2CH_3$	276	67	100
4	$C_6H_5C(=O)NHCH_2C(=S)SCH_3$	226	27	86
5	$CH_3C(=O)NHCH(CH_2C_6H_5)C(=O)NHCH_2C(=S)SCH_2CH_3$	325	37.4	132
6	$CH_3O)NHCH(CH_2C_6H_5)C(=O)NHCH_2C(=S)SCH_3$	299	27	130

mL H_2SO_4 in 5.85 mL ethanol and dissolved at room temperature. The solution was then placed in the ice-bath for 2–4 h. The precipitate was filtered, washed with cold ethanol, and dried under high vacuum overnight. The yield of amino acetonitrile sulphate, mp 152–153°C, was 89.7% (reaction [5]).

Amino acetonitrile salt (1.557 g, 0.01 mol) was dissolved in 13 mL of well-stirred 10% NaOH at the ice-bath temperature and, while stirring, 1.5 mL (0.0118 mol) *p*-chloro benzoylchloride in 3.5 mL THF was added over 20 min. The pH of the reaction was maintained at 14 by adding NaOH solution. After 3 h a quantitative amount of amido acetonitrile derivative was filtered and recrystallized from CCl_4. Further purification required dissolution in hot acetone, addition of charcoal, filtration through Celite 454, and precipitation of the final product with water (mp 145°C).

Results and discussion

The Pinner method of dithioester synthesis cannot be considered general for the preparation of this class of compounds, even though many attempts have been made. Using this approach, we tried to synthesize several dithioesters and the resulting yield was unsatisfactory (1), especially for the isotopic derivatives we wanted to make. Low yields in the classic Pinner procedure are a result of the poor solubility of N-substituted amino nitrile substrates in HCl saturated dichloromethane. Some of the N substitutions are not stable under these conditions especially in the presence of a trace amount of water. Use of liquid HF at low temperature gives full and immediate solubility of the N-substituted derivatives in the reaction mixture. This raises the reaction yield to the 70% range, which is a dramatic improvement over the old procedure (1). The final products synthesized by this improved method are summarized in Table 1. As an alternative, simple apparatus can be used: the reaction with HF could be performed in a polyethylene vessel, linked to the HF bottle on one side and a water aspirator on the other. This reactor must be capable of being cooled by liquid nitrogen and of being magnetically stirred. Such a simple arrangement for the reaction vessel could be considered as a general method for the synthesis of dithioesters of N-substituted amino acids and some peptides.

References

1. A.C. Storer, Y. Ozaki, and P.R. Carey. Can. J. Chem. **60**, 199 (1982).
2. K.I. Varughese, A.C. Storer, and P.R. Carey. J. Am. Chem. Soc. **106**, 8252 (1984).
3. P.R. Carey, H. Lee, Y. Ozaki, and A.C. Storer. J. Am. Chem. Soc. **106**, 8258 (1984).
4. H. Lee, A.C. Storer, and P.R. Carey. Biochemistry, **22**, 4781 (1983).
5. A.C. Storer, H. Lee, and P.R. Carey. Biochemistry, **22**, 4789 (1983).
6. K.M. Doyle and F. Kurzer. Chem. Ind. (London), 803 (1974).
7. D. Paquer. Bull. Soc. Chim. Fr. **5–6**, 1439 (1975).
8. C.S. Marvel, P. de Radzitzky, and J.J. Brader. J. Am. Chem. Soc. **77**, 5997 (1955).
9. S.R. Ramadas, P.S. Srinvasan, J. Ramachandran, and V.V.S.K. Sastry. Synthesis, 605 (1983).
10. A. Pinner and F. Klein. Ber. Dtsch. Chem. Ges. **11**, 1825 (1879).
11. J. Poupaert, A. Bruylants, and P. Crooy. Synthesis, 622 (1972).
12. R. West. J. Am. Chem. Soc. **79**, 4568 (1957); T.C. Waddington and F. Klanberg. J. Chem. Soc. 2329 (1960); D.W.A. Sharp. J. Chem. Soc. 2558 (1958).
13. W. König and R. Geiger. Chem. Ber. **103**, 788 (1970).