Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 11

SYNTHESIS OF 1-[^{11}C]-D,L-HOMOCYSTEINE THIOLACTONE: A POTENTIAL TRACER FOR MYOCARDIAL ISCHEMIA USING PET

KURT HAMACHER* and JAN HANUŠ

Institut für Chemie 1 (Nuklearchemie)
Kernforschungsanlage Jülich GmbH
D-5170 Jülich, Federal Republic of Germany

SUMMARY

The synthesis of 1-[^{11}C]-D,L-homocysteine thiolactone, a potential tracer for PET imaging of ischemic heart regions, is described. The labelling is achieved by reaction of [^{11}C]carbon dioxide with α-lithiated S-(tetrahydropyran-2-yl)3-thiopropylisonitrile. Deprotection of the mercapto group and lactonisation of the resulting thioamino acid is accomplished in an acid catalysed reaction. The radiochemical yield obtained is 10 to 15 % and the synthesis time, including the HPLC purification is about 45 min.

KEYWORDS: homocysteine thiolactone, carbon-11, S-(tetrahydropyran-2-yl)-3-thiopropylisonitrile, PET

INTRODUCTION

Homocysteine thiolactone is a metabolic heart agent which allows localization of regional limitations of impaired cardiac energy metabolism when the balance between myocardial oxygen demand and oxygen supply is upset[1]. A sensitive index for cardiac ischemia is the increased formation of adenosine due to the dephosphorylation of adenine nucleotides. The approach was to quantify regional changes in the free cytosolic concentration of this nucleoside via the enzymatic formation of S-adenosyl homocysteine (SAH) in the presence of ^{11}C-labelled homocysteine thiolactone. SAH accumulation

*To whom correspondence should be addressed

0362–4803/89/111275–09$05.00

Received May 12, 1989
Revised June 2, 1989

closely reflects steady state changes in the level of free cytosolic adenosine[2,3], and local changes in the free cardiac adenosine concentration of stenosed dog hearts can be assessed using positron emission tomography. First results indicate a positive imaging of ischemic heart regions and provide a potential method to localize regional myocardial ischemia[4].

Besides the labelling procedure which is based on the $[^{11}C]CO_2$ carboxylation method published by Vaalburg et al.[5] two alternative synthetic pathways for the precursor S-(tetrahydropyran-2-yl)3-thiopropylisonitrile are described.

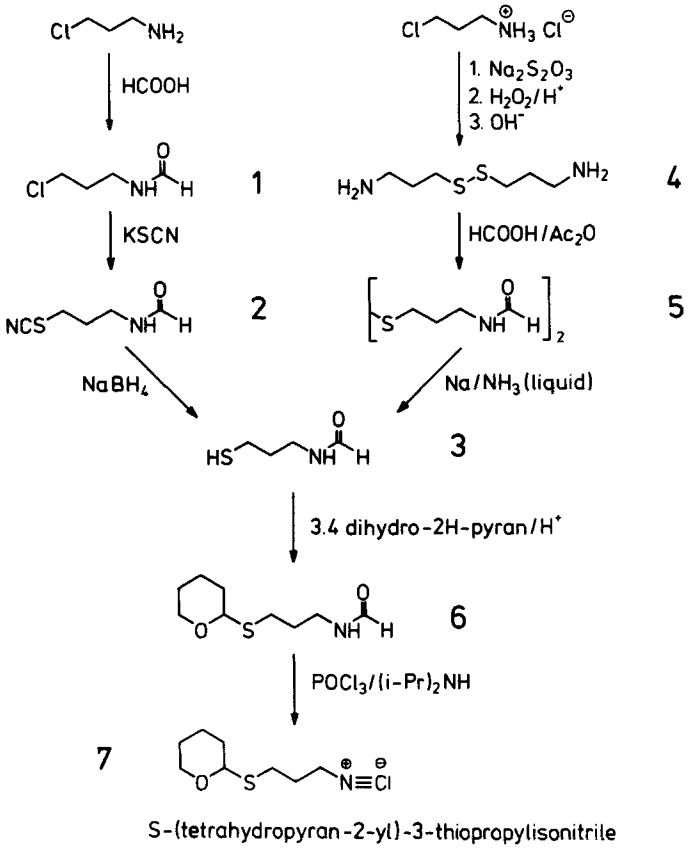
RESULTS AND DISCUSSION

The precursor tetrahydropyranyl thiopropylisonitrile (**7**) was prepared according to the following two alternative synthetic pathways as shown in scheme 1. The starting material in both cases was 3-chloropropylamine and the corresponding hydrochloride, respectively.

In the pathway A, starting with the formylation of the amine we obtained the 3-chloropropylformamide (**1**) via distillation with a chemical yield of 53 %. The formylation of the amine was follwed by the formation of 3-thiocyanatopropylformamide (**2**) via nucleophilic substitution using potassium thiocyanate. Reductive cleavage of the thiocyanato group by sodium tetrahydridoboranate in water solution led to the formation of 3-mercaptopropylformamide (**3**), the identical intermediate product of both synthetic alternatives.

The synthesis of bis(3-aminopropyl)disulfide (**4**), the primary product of pathway B was obtained via the intermediate Bunte salt. The crude reaction product was formylated using a mixture of formic acid and acetic acid anhydride in a chemical yield of about 33 %, based on the amount of 3-chloropropylamine. Cleavage of the disulfide does occur by sodium in liquid ammonia. The resulting 3-mercaptopropylformamide was not isolated but used without purification for the following synthetic step.

The synthetic pathway via the bis(3-aminopropyl)disulfide was more convenient because the reaction sequence via bis(3-formamidopropyl)disulfide (**5**) gave reasonably good yields (overall 10 %) and required only the chromatographic purification of compound **5**. In comparison with pathway B every synthetic step of the alternative way to compound **3** needed purification accompanied with diminishing of the chemical yield (overall 4 %). The preparation of S-(tetrahydropyran-2-yl)3-thiopropylformamide (**6**) does occur by the addition of the thiol to 3,4-dihydro-2H-pyran in the presence of catalytic amounts of hydrogen chloride[6] or Nafion-H, a superacidic perfluororesin sulfonic acid[7]. The yield of acetal formation was nearly the same in the case of homogeneous



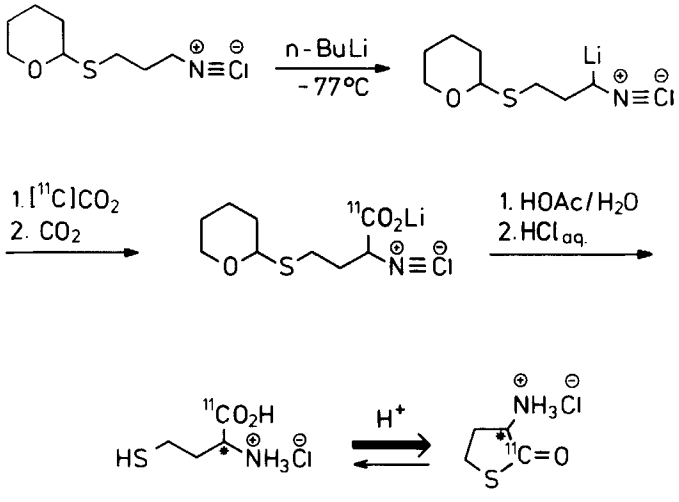
Scheme 1. Synthesis of the precursor **7** in a five step reaction with two different pathways to **3**.

and heterogeneous acid catalysis (52 %). The reason for using the tetrahydropyranyl residue as sulfhydryl protecting group is its excellent stability towards alkaline solutions which is necessary due to the strong basic conditions in the course of the C-11 carboxylation reaction. The reverse reaction, i.e. the hydrolytic cleavage of the semithioacetal to the mercaptane, is very rapid under acidic conditions. Accordingly, the acid catalysed formation of homocysteine after the preliminary step of $[^{11}C]CO_2$ insertion took place in a few minutes.

In the last step of precursor synthesis, the S-(tetrahydropyran-2-yl)-3-thiopropylisonitrile (**7**) was prepared by dehydration of the formamide by phosphoroxy trichloride in the presence of diisopropylamine[8]. To avoid polymerisation of the colourless and liquid product it was necessary to handle it under nitrogen or argon. Furthermore, it was relevant that the isonitrile did

not contain residual amounts of acids based on the incomplete basic hydrolysis of residual phosphoroxy chloride. The sensitive precursor was solubilized in tetrahydrofuran (distilled over $LiAlH_4$) and stored in inert atmosphere below 5°C in presence of molecular sieve. The ampoule was sealed by a teflon coated septum. The concentration of the stock solution was 0.17 mmol/ml.

The labelling procedure was based on the reaction of $[^{11}C]CO_2$ with α-lithiated isocyanide similar to the method developed by Vaalburg et al.[5]. The reaction sequence is shown in scheme 2. The isonitrile precursor was reacted with n-butyl lithium at about -77°C yielding a slightly yellow solution. The carboxylation was done in a two step sequence by carboxylation with $[^{11}C]CO_2$ followed by complete carboxylation of the residual lithium compound using carbon dioxide. Hydrolysation with the intermediate formation of N-formyl homocysteine and subsequent lactonisation took place in presence of 6 molar hydrochloric acid by heating under reflux for 8 to 10 minutes. Purification of the thiolactone is achieved by reversed phase chromatography using 0.1 molar sodium dihydrogenphosphate solution as eluent. The k'value of homocysteine thiolactone was 0.72 and the absolute retention time about 4 min. The radiochemical yield was in the range of 10 to 15 %. Up to 40 mCi $[^{11}C]$HCT were prepared, and the synthesis time, including HPLC purification, was 40 to 45 min E.O.B.



Scheme 2. Reaction sequence of radiotracer synthesis.

HPLC- and HPTLC-analysis of the purified carrier added $[^{11}C]$homocysteine thiolactone showed only one radioactive product which had the same retention time and R_f-value (0.38) as the unlabelled homocysteine thiolactone. The

chemical purity of the product was about 97 % and the radiochemical purity greater than 98 %.

EXPERIMENTAL

Thin layer chromatography was performed using E. Merck silica gel F254 plates and the spots were visualized under short wave UV-light and in the case of sulfur containing compounds with sodium nitroprusside reagent. Tetrahydropyranyl-thio derivatives were firstly sprayed with 1 molar hydrochloric acid, dried, sprayed with nitroprusside solution and alcalized with vapours of ammonia. The preparative purification of [^{11}C]homocysteine thiolactone via HPLC was carried out using a reversed phase column (Lichrosorb RP18, 250 x 10 mm) (MERCK) connected with a C-18 precolumn (20 x 4 mm). The eluent was sodium dihydrogenphosphate solution (0.1 mol/l) with a flow rate of 4.2 ml/min. The chromatographic device was equipped with a radioactivity monitor. IR-spectra (liquid films on KBr disks) were recorded on a Shimadzu infrared spectrophotometer IR-460. Flash chromatography was performed on silica gel column (3x23 cm) using silica gel 60 (70...230 mesh).

N-(3-chloropropyl)formamide 1

3-chloropropylamine hydrochloride (65 g, 0.5 mol) was solubilized in 150 ml methanol and neutralized with sodium hydroxide (20 g, 0.5 mol) in 300 ml methanol. The precipitated sodium chloride was filtered off and the solution evaporated in presence of formic acid (20 ml). The residue was suspended in benzene (500 ml) and stirred under reflux. After the formation of water was complete (about 3 h), the benzene was evaporated, the pale yellow oil diluted with ethanol (50 ml) and the solvent again removed. The product was purified by vacuum distillation yielding 21 g (34 %) colourless oil (bp. 103...107°C, $p < 10$ mbar).

N-(3-thiocyanatopropyl)formamide 2

A methanolic solution (20 ml) of **1** (5.3 g, 43 mmol) was treated with an excess of potassium thiocyanate (10 g, 0.1 mol) and was stirred under gentle reflux for about 24 h. The solution was evaporated to dryness and the residue diluted with 100 ml of chloroform. The product dissolved in chloroform was purified by flash chromatography. The formamide was eluted with chloroform yielding 3.4 g (67 %) of colourless oil. IR-spectroscopy: ν_S (SCN) 2165 cm^{-1}

N-(3-thiopropyl)formamide 3

N-(3-thiocyanatopropyl)formamide (4.1 g, 28 mmol) dissolved in 10 ml of water was added dropwise to a stirred solution of sodium borohydride (2.7 g, 0.07 mol) in 50 ml of water. Within 15 min, the temperature increased from 20 to 31°C. The solution was stirred for 2 h at 50°C and then allowed to cool to

room temperature. The solution was neutralized with about 25 ml of hydrochloric acid (2 mol/l) and then extracted 3 times with 50 ml dichloromethane. The organic phase was dried (anhydr. Na_2SO_4) and concentrated to a slightly yellow oil (1.5 g, 41 %). IR-spectroscopy showed no significant absorption in the range of 2165 cm^{-1} but an absorption band at 2250 cm^{-1} typical for thiols. TLC-analysis on silica gel plates ($CHCl_3$/MeOH, 10:1) showed almost pure thiol. R_f = 0.3...0.4, UV-detection and sodium iron (III) nitroso pentacyanide reagent.

Bis(3-aminopropyl)disulfide 4

3-chloropropylamine hydrochloride (11.7 g, 90 mmol) and sodium thiosulfate pentahydrate (22.3 g, 90 mmol) were dissolved in a mixture of 45 ml water and 25 ml methanol. The solution was refluxed for 3 h, cooled to 0°C and after adding hydrogen peroxide (12 ml, 30 % solution) the pH of the solution was adjusted to 1 with diluted sulfuric acid. The reaction mixture was placed in the refrigerator for 10 days. To accelerate the solubilization of the sedimented salt the mixture was shaken daily for some minutes. In the course of five days the salt normally dissolved. After standing for ten days in the refrigerator, excess of hydrogen peroxide was destroyed by stirring the cooled solution with a small amount of charcoal for 3 to 5 h. After filtration the solution was neutralized with sodium hydroxide and concentrated in vacuo to a volume of 20 to 30 ml. Sodium hydroxide (5.6 g, 140 mmol) was added and the salt suspension was extracted continuously with ethyl acetate (about 5 h). The organic phase was dried (anhydr. Na_2SO_4) and concentrated to dryness. The crude amine (5.35 g) obtained as a yellow oil was used for the next step without purification. TLC analysis (chloroform/ methanol/aq. ammonia 10:4:1) R_f = 0.2.

Bis(3-formamidopropyl)disulfide 5

An equimolar mixture of formic acid and acetic acid anhydride was heated to 60°C for 30 min and 20 ml of this formylation reagent slowly added to the solution of the crude amine **4** (5.35 g) dissolved in diethylether (20 ml). The clear solution was allowed to stand at room temperature for 4 h and then evaporated to dryness. The evaporation step was repeated twice after the addition of ethanol (2x10 ml). Water (5 ml) was added and residual acid neutralized with solid sodium hydrogen carbonate. When the carbon dioxide formation was complete, the organic phase was separated, dried (anhydr. Na_2SO_4) and evaporated to dryness. The crude amide (6.25 g) was purified by chromatography on silica gel column using chloroform/methanol (100:5) as eluent. When the first UV peak (254 nm) was observed (fraction 17) the eluent was changed to a volume ratio of 10:1. Fractions 17 to 50 (each fraction contained about 6 ml) were collected and contained pure product. The solution

was evaporated and to remove residual methanol, evaporation was repeated in presence of benzene (10 ml). The virtually colourless, viscous oil (3.45 g, 33 % based on chloropropylamine) contained only traces of impurity. TLC analysis (chloroform/methanol 10:1) R_f = 0.25

Elemental analysis: Calc. for $C_8H_{16}N_2S_2O_2$: C, 40.6 %; H, 6.88 %; N, 11.5 %
Found: C, 40.7 %; H, 6.83 %; N, 11.8 %

N-(3-thiopropyl)formamide 3

A solution of the disulfide **5** (2 g, 8.5 mmol) in 3 ml of tetrahydrofuran was mixed with 35 ml of liquid ammonia. To the stirred solution were added small pieces of sodium until the blue colour was steady for about 5 min. Excess of sodium was removed by the addition of ammonium chloride (blue colour disappears). The ammonia was allowed to evaporate and the salt residue evacuated at room temperature for 10 min and then for 10 min at 60°C. It is advantageous to scratch the solid out of the wall of the flask after evacuation at room temperature. Peroxide free ether (50 ml) was added to the salt and the suspension acidified with 3 molar hydrochloric acid to pH 2. The pink colour of the water layer disappeared when the solution became acidic. The two phase system was continuously extracted with ether for 2 h. The ether extract contained almost pure thiol 3 TLC (chloroform/methanol 10:1). R_f = 0.3...0.4

S-(tetrahydropyran-2-yl)-3-thiopropylformamide 6

The ether solution of thiol **3** was dried (anhydr. Na_2SO_4) and evaporated to a volume of approx. 20 ml. Some drops of dry ether solution saturated with hydrogen chloride were added with stirring followed by freshly distilled 3.4-dihydro-2H-pyran (10 ml). The pH of the solution (wet pH paper) was about 1.

After standing for 3 days at room temperature a solution of sodium hydroxide (10 g/100 ml) was added to neutralize the ether phase. The organic phase was separated, dried (anhydr. Na_2SO_4) and evaporated to dryness. A colourless oil (~ 4 g) was obtained and purified by silica gel column chromatography with chloroform/methanol (100:2) as eluent. The fraction collector was started when the first UV-peak (254 nm) was measured. Fractions 34 to 60 (6 ml per fraction) contained the pure product. Evaporation of the combined fractions yielded 2.7 g colourless and viscous oil (44 % based on the disulfide 4). TLC (chloroform/methanol 20:1) R_f = 0.3...0.4 Traces of an impurity could be detected with a somewhat higher R_f value.

Elemental analysis: Calc. for $C_9H_{17}NSO_2$ C, 53.2 %; H, 8.44 %; N, 6.80 %
Found: C, 52.5 %; H, 8.40 %; N, 6.40 %

Alternative pathway for the pyranylation of compound 3:
N-(3-thiopropyl)formamide (0.38 g, 1.6 mmol) and freshly distilled 3,4 dihydro-2H-pyran (0.3 ml) were dissolved in dichloromethane (5 ml) and in presence of Nafion-H (~ 100 mg) heated under reflux for 4 h. Nafion was filtered off and the solvent removed in vacuo. The crude oily product (~ 0.5 g) was chromatographed as described.

S-(tetrahydropyran-2-yl)-3-thiopropylisocyanide 7

At -10°C phosphoroxytrichloride (0.5 ml) was added dropwise (during 20 min) to a solution of **6** (1 g, 5 mmol) and diisopropylamine (1.86 ml) in 5 ml dichloromethane. The mixture was stirred at 0°C for 1 h. To the yellow solution was added $Na_2CO_3 \cdot 10H_2O$ (2.65 g) solubilized in 5 ml of water and then vigorous stirred at 25°C for 1 h. Subsequently, 5 ml of water and 3 ml dichloromethane were added to the suspension. After stirring for a few minutes the mixture was filtered, the organic phase separated and extracted twice with a small portion of water (2...3 ml). The dichloromethane solution was dried (anhydr. Na_2SO_4) and chromatographed on silica gel column with dichloromethane. The fractions 18 to 60 which contain the pure isonitrile were collected, evaporated under inert gas (Ar,N_2) and again evaporated in presence of 10 ml heptan for 3 times. A water-like colourless liquid was obtained (0.51 g, 55 %) and solubilized in THF (absol., dest. $LiAlH_4$) to get a stock solution with an isonitrile concentration of 0.17 mmol/ml. The solution was stored under Argon below 10°C in the presence of molecular sieve particles (3 Å).
TLC (chloroform/methanol 10:1) R_f = 0.65...0.7 IR, 2145 cm^{-1}, s

Carrier-added 1-[^{11}C]-D,L-homocysteine thiolactone

In a cylindrical reaction vessel 1 ml of the stock solution of S-(tetrahydropyran-2-yl)-3-thiopropylisonitrile (0.17 mmol) was diluted with 0.5 ml dry tetrahydrofuran, and while a stream of helium went through the solution, the vessel was chilled to about -77°C. Before the collection of the [^{11}C]CO_2 from the target gas flow was started, 80 µl n-butyl lithium in hexane (1.6 mol/l) was added dropwise to the isonitrile while helium bubbled continuously through the solution. The [^{11}C]CO_2 was transferred into the reaction flask and after finishing the [^{11}C]carboxylation (3...5 min) a gentle stream of carbon dioxide was led into the yellow solution. The colour disappeared immediately after carbon dioxide injection. The solution was transferred into a second reaction flask connected with an evaporator and evaporated to dryness at room temperature. A mixture of acetic acid/water (90:10) (1 ml) was added to the dry residue and mixed for 2 min at room temperature. Subsequently, 2 ml of hydrochloric acid (6 mol/l) was added and, after 1 min at room temperature, heated under reflux for 8 to 10 min. In the course of this step a gentle stream of helium went through the flask so that the

hydrolysis and lactonisation step was accompanied by a partial evaporation of the liquid phase. The remaining brown coloured solution was concentrated to a volume of about 0.5 ml and diluted with 1.5 ml sodium dihydrogenphosphate solution (0.1 mol/l) for the preparative HPLC purification.

The chemical and radiochemical purity of the [^{11}C]-homocysteine thiolactone was determined by TLC. The analyses were performed on RP 2 TLC plates (Merck) with a solvent mixture of equal parts by volume of isopentanol, dioxane, pyridine and water. The amino acid was detected by ninhydrin (R_f = 0.38).

ACKNOWLEDGEMENT

We thank Professor Dr. G. Stöcklin for his active support of this research programme and Ms. E. Assmann for technical assistance.

REFERENCES

1. Bardenheuer, H., and Schrader, J. - Am. J. Physiol. 250: H173 (1986)
2. Deussen, A., Borst, M., and Schrader, J. - Circ. Res. 63: 220 (1988)
3. Deussen, A., Borst, M., Kroll, K., and Schrader, J. - Circ. Res. 63: 250 (1988)
4. Deussen, A., Hamacher, K., Henrich, M., Borst, M., Walter, Ch., Herzog, H., Coenen, H.H., Stöcklin, G., Feinendegen L.E., and Schrader J. - Pflugers Archiv, Europ. J. Physiol. Suppl. No. 1 to 412: R27 (1988)
5. Vaalburg, W., Beerling van der Molen, H.D., Reiffers, S., Rijskamp, A., Woldring, M.G., and Wynberg, H. - J. of Applied Radiation and Isotopes 27: 153 (1976)
6. Kipnis, F., and Ornfelt, J. - J. Am. Chem. Soc. 73: 822 (1951)
7. Olah, G.A., Iyer S.P., Prakash, G.K.S. - Synthesis 513 (1986)
8. Obrecht, R., Herrmann, R., Ugi, I. - Synthesis Comm. 400 (1985)