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Synthesis of N-(2-[^{18}F]fluoroethyl)-N'-methylthiourea : a hydrogen peroxide scavenger

C. Gilissen, G. Bormans, T. de Groot and A. Verbruggen*

Laboratory of Radiopharmaceutical Chemistry F.F.W., K.U. Leuven, Herestraat 49
B-3000 Leuven, Belgium

SUMMARY

N-(2-[^{18}F]fluoroethyl)-N'-methylthiourea ([^{18}F]FEMTU), a fluorine-18 labelled derivative of the hydrogen peroxide scavenger dimethylthiourea (DMTU), has been synthesized by reaction of 2-[^{18}F]fluoroethylamine with methylisothiocyanate.
2-[^{18}F]Fluoroethylamine was obtained in modest radiochemical yields (39±6%, mean±sd, n=5, decay corrected) by nucleophilic substitution with [^{18}F]fluoride on N-[2-(*p*-toluenesulfonyloxy)ethyl]phthalimide followed by deprotection with hydrazine and distillation.
The distilled 2-[^{18}F]fluoroethylamine was trapped in CH_2Cl_2 and reacted with methyl-isothiocyanate to yield [^{18}F]FEMTU that was purified by reversed phase HPLC. The total synthesis takes 150 min and provides [^{18}F]FEMTU with a specific activity of 3.3±0.5 GBq/μmol (mean±sd, n=3) at end of synthesis, with an overall decay corrected radiochemical yield of 25±8% (mean±sd, n=5).

Key-words : DMTU, hydrogen peroxide, fluorine-18, oxidative stress

INTRODUCTION

An increasing number of studies demonstrate that oxidative stress is implicated in aging and several diseases, such as Parkinson's disease, Alzheimer's disease and multiple sclerosis (1). Oxygen-derived free radicals are formed during physiological metabolic reactions and pathological processes in tissues (2,3). The most reactive radical, namely the hydroxyl radical, is formed from superoxide and hydrogen peroxide by the Haber-Weiss reaction or from hydrogen peroxide in the presence of a cofactor (Fe^{2+} or Cu^{2+}) by the Fenton reaction (4). Normally, formation of these toxic species is prevented or regulated by conversion of superoxide to hydrogen peroxide

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by the enzyme superoxide dismutase, followed by conversion of hydrogen peroxide to H_2O and O_2 by either glutathione peroxidase or catalase. When these regulatory mechanisms are not completely effective, a pathological production of free radicals results (2). Free radicals alter the structure and function of lipids, proteins, polysaccharides and nucleic acids. The activity of membranes, enzymes and genetic material will be disrupted and the function of individual cells and various organs can be affected (4).

In vivo detection of free radicals would constitute an important tool for further research with regard to the role of free radicals in aging or disease and for the evaluation of potential therapies. Several methods for the *in vivo* detection of free radicals, such as electron spin resonance (ESR) (5) and chemiluminescence measurement (6) have been explored but the former offers only a low spatial resolution, whereas the latter is invasive. Single photon emission computerized tomography (SPECT) and positron emission tomography (PET) with radiolabelled derivatives of the free radical scavenger N-*tert*-butyl-α-phenylnitrone (PBN) have been proposed for the *in vivo* detection of free radicals (7-9). The amount of radiolabelled substances may, however, be too small to compete with cell lipids and proteins for the reaction with free radicals.

In vivo measurement of oxidative stress by detection of hydrogen peroxide could therefore offer an alternative to the former approach. Dimethylthiourea (DMTU) is a molecule which is frequently used as an *in vivo* scavenger of hydrogen peroxide, present in tissues exposed to oxidative stress (10). In the presence of hydrogen peroxide, DMTU is oxidized to DMTU-dioxide (11) which can be trapped intracellulary due to its increased polarity (Fig. 1). Thus, radioactive derivatives of DMTU could be potential probes for the localization of oxidative stress. For this reason, we have synthesized N-(2-[^{18}F]fluoroethyl)-N'-methylthiourea ([^{18}F]FEMTU), a fluorine-18 labelled derivative of DMTU.

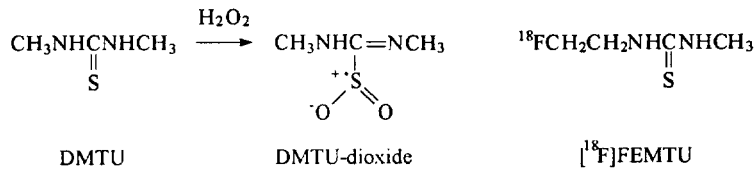

Fig. 1. Oxidation of DMTU with H_2O_2 yielding DMTU-dioxide and structure of [^{18}F]FEMTU

MATERIALS AND METHODS

Ethanolamine, di-*tert*-butyldicarbonate, triethylamine, *p*-toluenesulfonyl chloride, tetraethylammonium fluoride dihydrate, potassium phthalimide, hydrazine monohydrate, phenylhydrazine, potassium carbonate and Kryptofix 2.2.2 were obtained from Acros Chimica. 2-Fluoroethylamine hydrochloride, ethyleneglycol di-*p*-tosylate and methylisothiocyanate were obtained from the Aldrich Chemical Company and were used without further purification. Reaction residues were purified by column chromatography on silica gel (Silica gel 60, Merck, Darmstadt, Germany). ^{1}H-NMR spectra were recorded on a Varian 200 MHz spectrometer. Chemical shifts are reported in ppm relative to TMS (δ=0). Liquid secondary ion mass spectra (LSIMS) were recorded on a Kratos Concept mass spectrometer (Kratos Analytical, Manchester, UK) connected with a Masspec II data system (MSS, Ltd, Manchester, UK). Melting points were determined in open capillaries immersed in an oil bath (Büchi-Tottoli) and are not corrected. [^{18}F]Fluoride was produced by irradiation of 90% enriched [^{18}O]water (Isotec, OH, USA) with 10-MeV protons using a Cyclone 10/5 cyclotron (Ion Beam Applications, Louvain-la-Neuve, Belgium).

Solutions were evaporated in a modified microwave oven (Bosch HMT 600 c, Berlin, Germany) at 650 Watt. HPLC analyses were performed on a 250 mm x 4.6 mm Hypersil BDS C18 column (Alltech, Laarne, Belgium) eluted with H_2O/EtOH (95:5 V/V) at a flow rate of 1 ml/min (system A) or with gradient mixtures of 0.025 M

phosphate buffer pH 5.85 (A) and EtOH (B) (t=0 min: 85% A; linearly decreased to 50% A at t=20 min) at a flow rate of 1 ml/min (system B). [^{18}F]FEMTU was purified on a 250 mm x 10 mm Hypersil ODS C18 column (Alltech, Laarne, Belgium) eluted with H_2O/EtOH (95:5 V/V) at a flow rate of 3 ml/min (system C). Radioactivity in the column eluate was detected with a 4-in. NaI(Tl) scintillation detector. UV absorbance was detected at 240 nm. The UV absorbance and radiometric detection signals were fed into a Rachel integration system (LabLogic, Sheffield, UK). Retention times of the different compounds are presented in Table 1.

Table 1. RP-HPLC retention times (sec)

compound	system A	system B	system C
N-*t*-BOC-[2-(*p*-toluenesulfonyloxy)ethylamine] (1)	-	1630	-
N-*t*-BOC-(2-fluoroethylamine) (2)	-	1090	-
N-[2-(*p*-toluenesulfonyloxy)ethyl]phthalimide (4)	-	1270	-
N-(2-fluoroethyl)phthalimide (5)	-	925	-
N-(2-fluoroethyl)-N'-methylthiourea (6)	380	-	710

Synthesis of intermediates and final product

N-t-BOC-2-aminoethanol (19)

A solution of ethanolamine (6.1 g, 100 mmol) in 150 ml dichloromethane was cooled to 0°C and triethylamine (15.25 ml, 110 mmol) was added. Di-*tert*-butyl dicarbonate (21.8 g, 100 mmol) dissolved in 20 ml dichloromethane was added and the solution was stirred overnight at room temperature. The solution was extracted with water (100 ml), dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield an oil (10 g, 62%).

^{1}H-NMR ($CDCl_3$) : δ 1.46 (s, 9H), 3.16 (q, 2H), 3.57 (q, 2H), 5.23 (s, 1H).

N-t-BOC-[2-(p-toluenesulfonyloxy)ethylamine] (1)

A solution of N-*t*-BOC-2-aminoethanol (1 g, 6.2 mmol) in 10 ml dichloromethane was cooled to 0°C and triethylamine (0.95 ml, 6.85 mmol) was added. After addition of *p*-toluenesulfonyl chloride (1.18 g, 6.2 mmol) the solution was stirred overnight at

room temperature, washed with water (20 ml), dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography with hexane/ethyl acetate (8:2 V/V) as the eluent to yield 0.6 g (30.7%) of N-*t*-BOC-[2-(*p*-toluenesulfonyloxy)ethylamine].

^{1}H-NMR ($CDCl_3$) : δ 1.46 (s, 9H), 2.45 (s, 3H), 3.39 (q, 2H), 4.33 (t, 2H), 7.32 (d, 2H), 7.74 (d, 2H); mp 63-65°C.

N-t-BOC-(2-fluoroethylamine) (2)

To a solution of N-*t*-BOC-[2-(*p*-toluenesulfonyloxy)ethylamine] **(1)** (2 g, 6.35 mmol) in 30 ml acetonitrile was added tetraethylammonium fluoride dihydrate (1.177 g, 6.35 mmol), dissolved in 50 ml acetonitrile. The solution was heated at 70°C for 10 minutes and then concentrated under reduced pressure. The reaction product was purified by column chromatography with hexane/ethyl acetate (8:2 V/V) as the eluent to yield **2** (0.8 g, 77%) as an oil.

^{1}H-NMR ($CDCl_3$) : δ 1.46 (s, 9H), 3.23 (dm, 2H, $^3J_{HF}$=24Hz), 4.35 (dt, 2H, $^2J_{HF}$ =48Hz).

N-[2-(p-toluenesulfonyloxy)ethyl]phthalimide (4)

Ethyleneglycol di-*p*-tosylate (10 g, 27 mmol) was dissolved in 50 ml DMF and potassium phthalimide (5 g, 27 mmol) was added with stirring. The mixture was stirred at 100°C for 2 hours. The solution was cooled to room temperature, poured into water (50 ml) and extracted with dichloromethane (2 x 50 ml). The dichloromethane layer was washed with water (100 ml), dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography with hexane/ethyl acetate (4:3 V/V) as the eluent to yield N-[2-(*p*-toluenesulfonyloxy)ethyl]phthalimide (4.2 g, 45%) as white crystals.

^{1}H-NMR ($CDCl_3$) : δ 2.35 (s, 3H), 3.62 (t, 2H), 4.37 (t, 2H), 7.32 (d, 2H), 7.53 (d, 2H), 7.9 (d, 2H) ; mp 143-144°C (Lit 144.5°C,(13)).

N-(2-fluoroethyl)phthalimide (5)

N-[2-(*p*-Toluenesulfonyloxy)ethyl]phthalimide (4) (0.2 g, 0.57 mmol) was dissolved in 25 ml acetonitrile and tetraethylammonium fluoride dihydrate (0.12 g, 0.65 mmol), dissolved in 50 ml acetonitrile was added with stirring. The solution was heated at 70°C for 10 minutes and evaporated under reduced pressure. The residue was purified by column chromatography with hexane/ethyl acetate (6:4 V/V) as the eluent to yield N-(2-fluoroethyl)phthalimide (0.08 g, 71%) as a white powder.

^{1}H-NMR ($CDCl_3$) : δ 4.02 (dt, 2H, $^3J_{HF}$ =24 Hz), 4.46 (dt, 2H, $^2J_{HF}$ =48 Hz), 7.53 (d, 2H), 7.9 (d, 2H); mp 97-99°C (Lit 97-100°C,(14)).

N-(2-fluoroethyl)-N'-methylthiourea (FEMTU) (6)

A solution of methylisothiocyanate (0.37 g, 5 mmol) in 5 ml dioxane was cooled to 0°C and K_2CO_3 (0.35 g, 2.5 mmol) and 2-fluoroethylamine hydrochloride (0.5 g, 5 mmol) were added with stirring. The resulting precipitate was filtered off. The filtrate was evaporated under reduced pressure and the residue was purified by column chromatography with water/ethanol (95:5 V/V) as the eluent to yield 0.28 g (41%) of FEMTU as an oil.

^{1}H-NMR (DMSO) : δ 2.99 (d, 3H), 3.8 (dm , 2H, $^2J_{HF}$ =24 Hz), 4.5 (dt, 2H, $^3J_{HF}$ =48 Hz), 5.3 (s, 2H). LSIMS (thioglycol) $[M+H]^+$ 137.

2-[^{18}F]fluoroethylamine (3)

After irradiation of 400 µl [^{18}O]water with 10-MeV protons for 30 minutes at a beam current of 20 µA, the contents of the target were passed over a 4-mm diameter ion exchange membrane (AG1-X8, OH^- form, Bio-Rad, CA, USA) to trap [^{18}F]fluoride. The membrane was rinsed with 300 µl H_2O and [^{18}F]fluoride was then eluted with a solution of 5 mg Kryptofix 2.2.2 and 0.5 mg K_2CO_3 in 500 µl 90% aqueous methanol (12). The solution was evaporated in a microwave oven. Then, two 200-µl aliquots

of absolute ethanol were added and each time evaporated to remove traces of water (12). The residue containing [^{18}F]fluoride, Kryptofix 2.2.2 and K_2CO_3 was then used in one of the following procedures:

Method A

In a typical run, N-*t*-BOC-[2-(*p*-toluenesulfonyloxy)ethylamine] (**1**, 5 mg) dissolved in 500 µl anhydrous acetonitrile was added to the [^{18}F]fluoride residue, containing 290 MBq [^{18}F]fluoride, and the solution was heated for 5 minutes in an oil bath at 70°C. The reaction mixture was passed over a C18 SepPak column (Waters Corporation, Massachusetts, USA) that was rinsed with 6 ml of H_2O to remove unreacted [^{18}F]fluoride. N-*t*-BOC-(2-[^{18}F]fluoroethylamine) (64 MBq, decay corrected) was then eluted with 2 ml of ethanol. The amino group was deprotected by addition of 0.1 ml of a 33% m/V solution of hydrogen bromide in glacial acetic acid and incubation for 10 minutes at room temperature. The mixture was then basified with 0.3 ml NaOH 6 N to obtain 64 MBq 2-[^{18}F]fluoroethylamine, as confirmed by reversed phase HPLC (RP-HPLC) analysis of the reaction mixture.

Method B

N-[2-(*p*-Toluenesulfonyloxy)ethyl]phthalimide (**4**, 5 mg), dissolved in 500 µl anhydrous acetonitrile, was added to the [^{18}F]fluoride residue, containing 140 MBq [^{18}F]fluoride, and the solution was heated in a microwave oven so that a pressure of 40-60 kPa was maintained for 5 minutes. The acetonitrile was subsequently evaporated under a stream of nitrogen. Hydrazine monohydrate (100 µl) was then added to the residue and 66 MBq 2-[^{18}F]fluoroethylamine was obtained.

N-(2-[^{18}F]fluoroethyl)-N-methylthiourea ([^{18}F]FEMTU) (6)

The formed 2-[^{18}F]fluoroethylamine (boiling point 63°C (16)) was distilled, with a yield of 13±1% (2±1% of [^{18}F]fluoride activity at EOB, method A) or 61±8% (39±6%

of [^{18}F]fluoride activity at EOB, method B), from the reaction mixture at 70°C with a stream of nitrogen into a solution of 10 mg (method A) or 150 mg (method B) methylisothiocyanate in 2 ml dichloromethane and the solution was incubated at room temperature for 40 minutes. The reaction mixture was evaporated under a stream of nitrogen, the residue dissolved in 1 ml water and purified by RP-HPLC (system C). Overall decay corrected yields were 1±1% (mean±sd, n=6) (method A) and 25±8% (mean±sd, n=5) (method B) (Table 2).

Table 2. Yields of intermediate products based on [^{18}F]fluoride activity (decay corrected to EOB)

method A		method B	
$^{18}F^-$ (100-400 MBq)		$^{18}F^-$ (140-200 MBq)	
↓		↓	
N-*t*-BOC-(2-[^{18}F]fluoroethylamine)	21±9%	N-(2-[^{18}F]fluoroethyl)phthalimide	56±13%
↓		↓	
distilled 2-[^{18}F]fluoroethylamine	2±1%	distilled 2-[^{18}F]fluoroethylamine	39±6%
↓		↓	
[^{18}F]FEMTU (0.5-8 MBq)	1±1%	[^{18}F]FEMTU (35-65 MBq)	25±8%

Determination of the specific activity

Specific activity was calculated for the intermediate N-(2-[^{18}F]fluoroethyl) phthalimide, which has a good chromophore at 254 nm, and decay corrected to the end of synthesis of [^{18}F]FEMTU. The specific activity was determined using a calibration curve obtained by UV absorption (254 nm) of nonradioactive authentic N-(2-fluoroethyl)phthalimide.

RESULTS AND DISCUSSION

N,N'-substituted thiourea derivatives can generally be synthesized by reaction of an amine with methylisothiocyanate (17,18). A first approach to synthesize N-(2-[^{18}F]fluoroethyl)-N'-methylthiourea was to prepare a precursor with a good leaving

group from which the intended tracer agent could be obtained in a single step by a nucleophilic substitution reaction with [^{18}F]fluoride. Such precursor would be N-2-(*p*-toluenesulfonyloxy)ethyl-, N-2-iodoethyl- or N-2-bromoethyl-N'-methylthiourea. Unfortunately, the reaction of 2-(*p*-toluenesulfonyloxy)ethylamine or 2-bromoethylamine with methylisothiocyanate failed to give the expected precursor and instead, a cyclic product was formed (Fig.2) as confirmed with ^{1}H-NMR analysis.

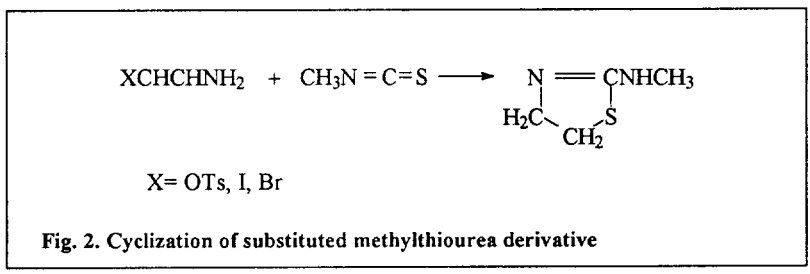

Fig. 2. Cyclization of substituted methylthiourea derivative

As we were unsuccessful in preparing a precursor for a one-step synthesis, we changed our strategy to a three-step procedure for the preparation of [^{18}F]FEMTU (Fig. 3). Two pathways for the synthesis of 2-[^{18}F]fluoroethylamine were explored.

In the first approach 2-[^{18}F]fluoroethylamine was obtained with a radiochemical yield of 21±9% (mean±sd, n=6, decay corrected) by nucleophilic substitution of N-*t*-BOC-[2-(*p*-toluenesulfonyloxy)ethylamine] (**1**) with [^{18}F]fluoride followed by removal of the *t*-BOC protecting group with hydrogen bromide and basification. Under these conditions, only a limited fraction of the formed 2-[^{18}F]fluoroethylamine, namely 13±1% (2±1%, mean±sd, n=6, decay corrected, based on [^{18}F]fluoride activity) could be distilled from the reaction vessel. Of the distilled 2-[^{18}F]fluoroethylamine, 47±8% (mean±sd, n=6) reacted with methylisothiocyanate to yield [^{18}F]FEMTU that was purified by RP-HPLC (system C). [^{18}F]FEMTU was obtained with an overall decay corrected yield of 1±1% (mean±sd, n=6) after 150 minutes of synthesis.

A higher overall yield and a better reproducibility were obtained with our second approach to the synthesis of [^{18}F]FEMTU, using in a first step the recently reported method of Tewson (15), to prepare N-[2-(*p*-toluenesulfonyloxy)ethyl]phthalimide (**4**).

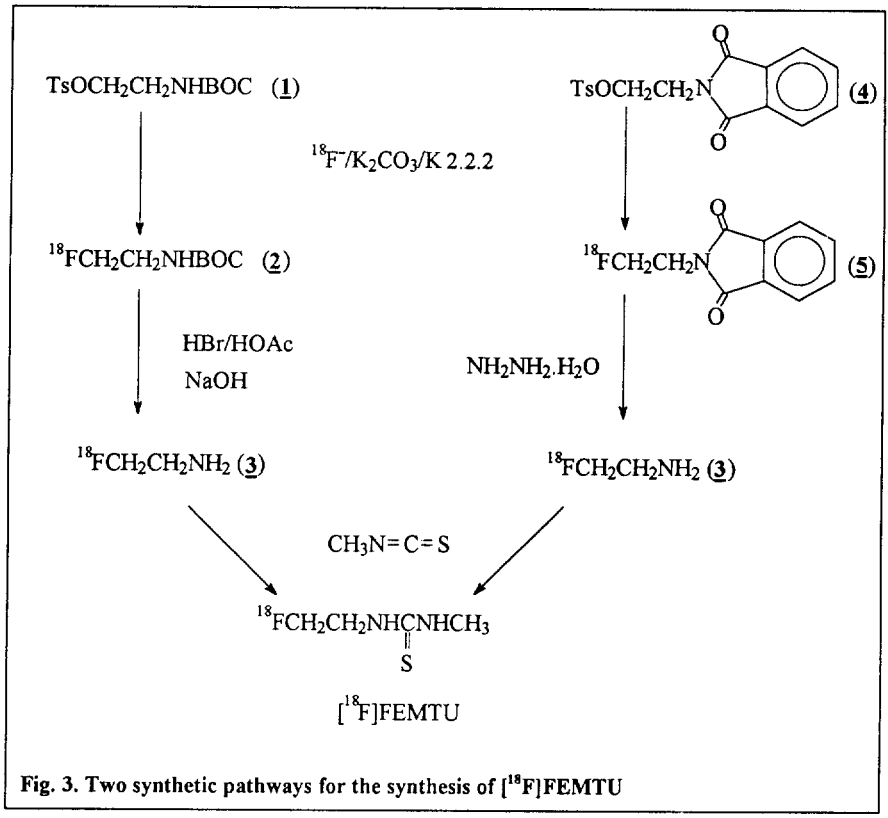

Fig. 3. Two synthetic pathways for the synthesis of [^{18}F]FEMTU

2-[^{18}F]Fluoroethylamine was obtained with a radiochemical yield of 56±13% (mean±sd, n=5, decay corrected) by nucleophilic substitution of **4** with [^{18}F]fluoride. After evaporation of acetonitrile, N-(2-[^{18}F]fluoroethyl)phthalimide **5** was deprotected with hydrazine monohydrate. After the hydrazinolysis, the formed 2-[^{18}F]fluoroethylamine could be distilled with a yield of 61±8% (39±6%, mean±sd, n=5, decay corrected, based on [^{18}F]fluoride activity) at 70°C and trapped in dichloromethane where it was reacted with methylisothiocyanate at room temperature. When phenylhydrazine was used for the deprotection of the amino group, only 9±2% (mean±sd, n=3, decay corrected) of the formed 2-[^{18}F]fluoroethylamine could be distilled. The large excess of methylisothiocyanate with respect to 2-[^{18}F]fluoroethylamine was necessary because hydrazine co-distils and reacts

competitively with methylisothiocyanate. A 40-min reaction time was necessary to complete the reaction, after which the organic solvent had to be removed to allow purification by RP-HPLC.

Of the distilled 2-[^{18}F]fluoroethylamine, 90±7% (mean±sd, n=5) was converted to [^{18}F]FEMTU as identified by co-elution with authenthic FEMTU on RP-HPLC. [^{18}F]FEMTU appeared unstable in solution and slowly decomposed as a function of time. After storage of [^{18}F]FEMTU for 1 hour at room temperature, 63% of the tracer product was still in intact form. This decomposition could be prevented by addition of 3 mg ascorbic acid. After storage for 2 hours in the presence of ascorbic acid, the tracer was still completely in intact form.

[^{18}F]FEMTU was obtained with a decay corrected yield of 25±8% (mean±sd, n=5) with a specific activity of 3.3±0.5 GBq/μmol (mean±sd, n=3) at end of synthesis. The total synthesis takes 150 minutes. This route provides sufficient amounts of [^{18}F]FEMTU to allow animal evaluation which is in progress and will be reported in a separate paper.

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