^{14}C-labelling of a Novel Antiischaemic Adenosine A_1 agonist at purine C-8.

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SUMMARY

A ^{14}C-labelled form of 2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine (1), a novel antiischaemic adenosine A_1 agonist, has been prepared in three steps from [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (2). The overall radiochemical yield was 56%. The radiochemical purity was higher than 98% with a specific radioactivity of 36 mCi/mmol.

Key words: ^{14}C, adenosine A_1 agonist, 2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine.

INTRODUCTION

Adenosine is a naturally occurring purine nucleoside which modulates synaptic transmission in the central nervous system (CNS) without itself acting as a neurotransmitter[1]. It is becoming apparent that adenosine and adenosine receptor ligands have a range of potential applications in the therapy of CNS disorders[2], since adenosine appears to act as as an endogenous neuroprotectant[3,4] and antiepileptic agent[5,6].

Adenosine receptors represent a subclass (P_1) of the group of purine nucleotide and nucleoside receptors known as purinoreceptors. The main pharmacologically distinct adenosine receptor subtypes are known as A_1, A_{2a}, A_{2b} (of high and low affinity) and A_3[7]. Selective ligands exist for these adenosine receptors, and the structure-activity relationships of the various reference ligands have been reviewed[8].

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Among the known adenosine receptor agonists most selective for the A_1 receptor over the A_2 receptor are the compounds where the adenine nucleus is substituted with a cycloalkyl group on the amino function, for example *N*-cyclopentyladenosine[9] (CPA) or 2-chloro-*N*-(1-piperidinyl)adenosine[10] (NNC 90-1515). However, a novel series of adenosine A_1 receptor agonists with potent CNS effects but with lowered cardiovascular effects has recently been identified[6,11]. In order to facilitate the pharmacological, pharmacokinetic and metabolic examination of 2-chloro-*N*-[(R-)(2-benzoxazolyl)thio-2-propyl]-adenosine, a member of this novel series of compounds, a suitable radioligand was required.

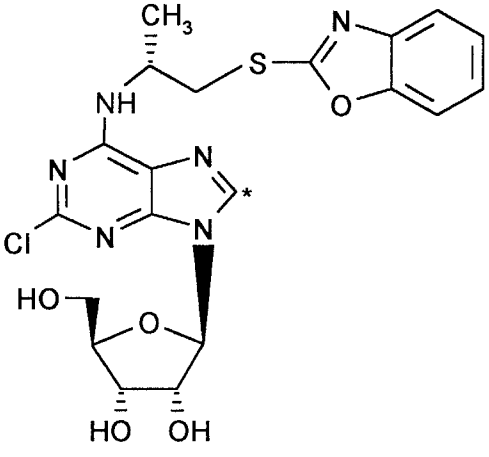
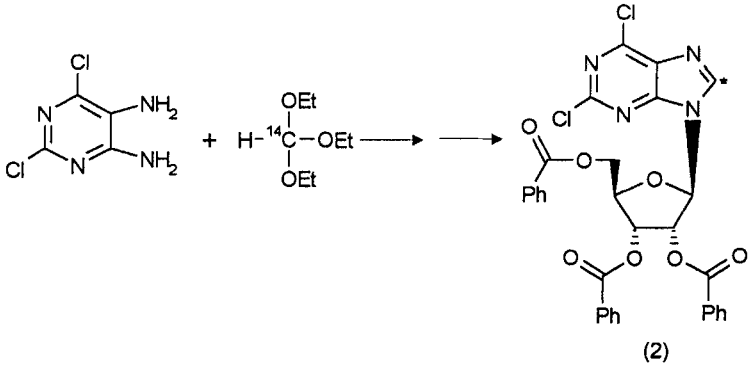


Figure 1 Structure of 2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine. * Indicates the position of ^{14}C labelling.

The preferred isotope for such experiments is ^{14}C, located in a stable part of the molecule; ^{14}C has a long half-life and a suitable energy of decay, and ^{14}C cannot be exchanged *in vivo*.

A general and efficient method for incorporation of ^{14}C into the 8-position of the purine heterocycle has previously been described by Valsborg *et al.*[12], using triethyl

[^{14}C]orthoformate as the radioactive starting material (Scheme 1). In the present work, our approach was to use [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (2) as the preferred radioactive starting material in the synthesis of ^{14}C-labelled 2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine.



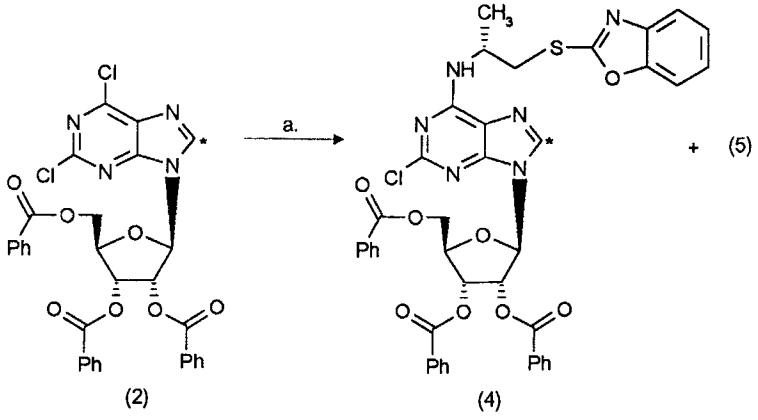
(2)

Scheme 1 Synthesis of [8-^{14}C]-2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (2) using 4,5-diamino-2,6-dichloropyrimidine as starting material[12].

RESULTS AND DISCUSSION

The first approach was to synthesize [8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]-adenosine (4) according to Scheme 2 (method A). Optimization studies using 2 with low specific radioactivity showed that the target structure (4) and a by-product (5) were formed in equal amounts when 2 was reacted with (R)-1-(2-benzoxazolylthio)-2-propylamine hydrochloride (3) in dioxane in the presence of triethylamine.

Analysis of 5 by mass spectrometry indicated that it had a molecular weight identical to 4. It was observed that the amine 3 was apparently unstable under the reaction conditions described above. This is probably the reason for the formation of two products. Owing to this fact and the consistently low labelling yield, another synthetic route was selected (Scheme 3, method B).



a. (R)-1-(2-benzoxazolyl)thio-2-propylamine hydrochloride (3), Et_3N, dioxan.

Scheme 2 Synthesis of [8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]-adenosine (4) using (R)-1-(2-benzoxazolylthio)-2-propylamine hydrochloride (3) as amine (method A).

Reaction of 2 with (R)-2-amino-1-propanol in dioxane with triethylamine as base produced 6 in a labelling yield of about 90% in trials using low specific radioactivity. The alcohol of 6 was used directly without purification and reacted with 2-mercaptobenzoxazole in a Mitsunobu reaction[13] leading to 4. The resulting protected nucleoside 4 could be isolated by preparative straight phase HPLC as a colorless solid. The overall labelling yield was about 68%, as determined by radio-TLC.

The HPLC-radiochromatograms from representative syntheses of 4 using method A and method B, are shown in Figure 2. As mentioned above, method A results in a by-product (5) which is not observed using method B.

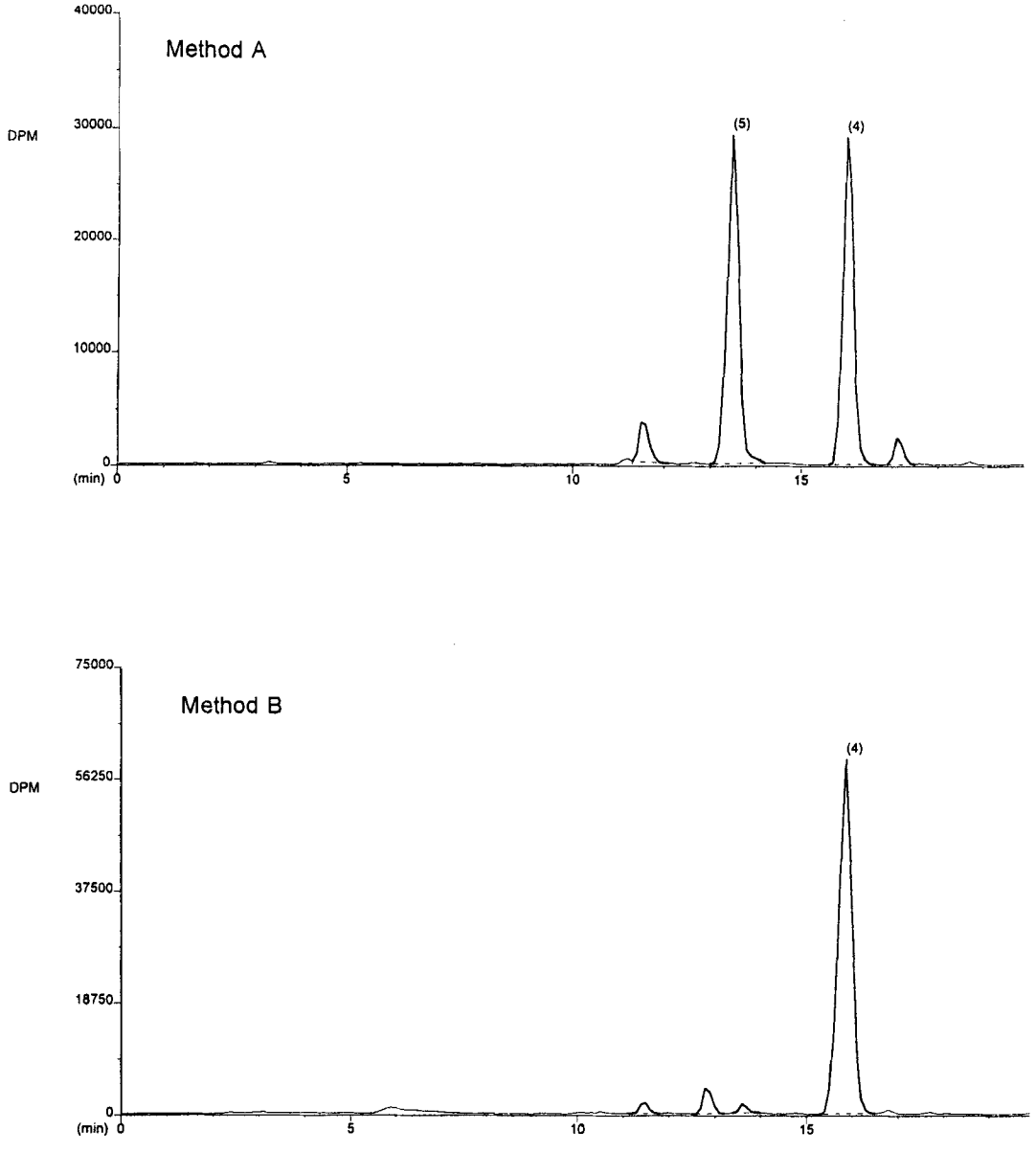
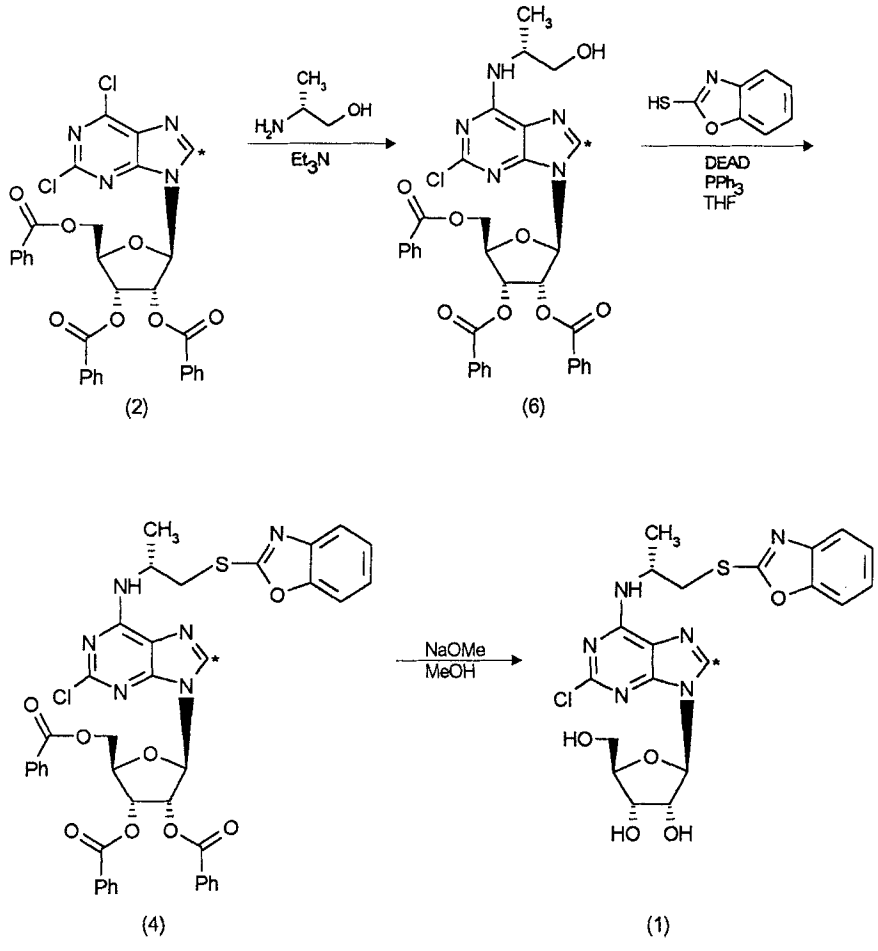


Figure 2 HPLC-radiochromatograms of ^{14}C-labelled [8-^{14}C]-2',3',5'-tri-O-benzoyl-2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine (4) and 5 (unknown) using synthetic method A and method B, respectively.



Scheme 3 Synthesis of [8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine (4) using (R)-2-amino-1-propanol as amine (method B) and deprotection of 4 with methoxide.

The optimized reaction conditions (method B) were used in the synthesis of 4 with high specific radioactivity, resulting in 60% overall radiochemical yield with a radiochemical purity >98%.

In optimization studies, methoxide in methanol proved to be superior to ammonia in methanol as the debenzoylation reagent. Thus, deprotection of 4 with methoxide gave 2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine (1) in 94% radiochemical yield from 4 (Scheme 3).

In conclusion, [8-^{14}C]-2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine was synthesized in 3 steps, starting from [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (2). The overall radiochemical yield was 56%, the radiochemical purity >98% and the specific radioactivity was 36 mCi/mmol.

EXPERIMENTAL

Optimization studies:

Material.

To a vial containing [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (50 μCi, 0.0014 mmol, specific activity 36 mCi/mmol) was added 122 mg of non-radioactive 9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine[10], and the solids were dissolved in 1000 μl dioxane. The specific radioactivity of this "spiked" solution was 0.26 mCi/mmol.

Procedures using low specific activity:

[8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine (4)
Method A.

To a vial containing [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (10 μCi, 0.039 mmol), (R)-1-(2-benzoxazolylthio)-2-propylamine) hydrochloride (3) (58.90 mg, 0.200 mmol) and triethylamine (87.90 μl, 0.634 mmol) was added dioxane (300 μl). The mixture was stirred at room temperature, and the reaction was followed by taking out samples (approximately 1 μCi) at different intervals. The labelling yields were determined using radio-HPLC and a reference standard. After 21 hours, less than 5% of the starting material remained and two products were formed in equal amounts (approximately 40% labelling yield each).

[8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-1-hydroxy-2-propyl]adenosine (6)
(R)-2-Amino-1-propanol (1.75 μl, 0.024 mmol) and triethylamine (3.73 μl, 0.027 mmol) were dissolved in dioxan (300 μl). [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (10 μCi, 0.039 mmol) was introduced. The vial was sealed and the mixture was stirred at room temperature. The reaction was followed by taking out samples (approximately 1 μCi) at different intervals. The labelling yields were determined using radio-HPLC and radio-TLC. After 17 hours, less than 5% of the starting material was left and a new product was formed in 80-93% labelling yield. The product was used without further purification in the synthesis of (4).

[8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine (4) Method B.

[8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-1-hydroxy-2-propyl]adenosine (6) (10 µCi, 0.036 mmol) was dissolved in dry THF (1 ml). Triphenyl phosphine (20.90 mg, 0.080 mmol), 2-mercaptobenzoxazole (8.08 mg, 0.053 mmol) and diethyl azodicarboxylate (12.4 µl, 0.080 mmol) were introduced. The mixture was stirred at room temperature. The reaction was followed by taking out samples (approximately 1 µCi) at different intervals. The labelling yields were determined using radio-HPLC and reference standard. After 3-4 hours all the starting material was reacted and (4) was formed in about 75% labelling yield.

Procedures using high specific activity:

[8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-1-hydroxy-2-propyl]adenosine (6)

[8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine (800 µCi, 0.024 mmol) was dissolved in dioxane (200 µl). (R)-2-amino-1-propanol (1.75 µl, 0.024 mmol) and triethylamine (3.73 µl, 0.027 mmol) were introduced and the vial was sealed. The mixture was stirred for 44 hours at room temperature. The solvent was removed by nitrogen flow, and the crude product was used without further purification in the synthesis of 4.

Radiochemical yield: 640 µCi (80%), determined by radio-HPLC (system A). The identity of 6 was determined by HPLC using a reference standard.

[8-^{14}C]-2',3',5'-tri-*O*-benzoyl-2-chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine (4)

Crude 6 (640 µCi, 0.018 mmol) was dissolved in dry THF (300 µl). Triphenyl phosphine (15.30 mg, 0.058 mmol), 2-mercaptobenzoxazole (6.99 mg, 0.046 mmol) and diethyl azodicarboxylate (9.0 µl, 0.058 mmol) were added. The mixture was stirred at room temperature for 24 hours. The volatiles were removed by nitrogen flow and the crude product was dissolved in a mixture of ethyl acetate, heptane and methanol (50/45/5, 2 ml) and purified on a semi-preparative straight phase HPLC column (250 x 20 mm, 15 µm) using a mixture of ethyl acetate and heptane (50/50) as eluent. The collected fractions were concentrated by evaporation to afford a colorless solid.

Radiochemical yield: 480 µCi (75%). Radiochemical purity >98%, determined by radio-TLC (system I) and radio-HPLC analysis (system A). The identity of 4 was determined by HPLC using a reference standard.

A nonradioactive sample of 4 was prepared using similar reaction conditions. ^{1}H-NMR (DMSO-d_6): 1.38 (3H, d, CH_3); 3.47-3.77 (2H, 2 x dd, CH_2); 4.62-4.89 (3H, m, H-4' +

H-5'$_a$ + H-5'$_b$); 6.19 (1H, t, H-3'); 6.34 (1H, t, H-2'); 6.52 (1H, d, H-1'); 7.28 (2H, m); 7.49 (2H, m); 7.52-7.98 (15H, m, OBz); 8.40 (1H, s, CH); 8.57 (1H, d, NH).

[8-^{14}C]-2-Chloro-*N*-[(R)-(2-benzoxazolyl)thio-2-propyl]adenosine (1)

The ^{14}C-labelled nucleoside 4 (480 µCi, 0.013 mmol) was dissolved in 1,5 ml of methanol. A solution of sodium methoxide (60 µl, 1.9 mg sodium/ml methanol) was added, and the mixture was stirred at room temperature. After 9 hours, radio-HPLC indicated >95% conversion. The solvent was removed by evaporation. The crude product was dissolved in a mixture of acetonitrile and water (70/30) and purified on a semi-preparative C-18 HPLC column (250 x 25 mm, 10 µm) using a mixture of water and acetonitrile (65/35) as eluent. The collected fractions were concentrated by evaporation to give a colorless solid.

Radiochemical yield: 451 µCi (94%), determined by radio-HPLC. Radiochemical purity >98%, determined by radio-HPLC analysis (system B) and radio-TLC (system II). The specific radioactivity was 36 mCi/mmol, determined by MS.

MATERIALS

All solvents used were of analytical grade. [8-^{14}C]-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloropurine was synthesized in our laboratory as previously described[12]. (R)-2-amino-1-propanol was obtained from Sigma, USA. Diethyl azodicarboxylate (DEAD) was obtained from Aldrich-Chemie, Germany. Triphenyl phosphine and 2-mercaptobenzoxazole were obtained from Fluka, Switzerland. (R)-1-(2-benzoxazolylthio)-2-propylamine) hydrochloride was synthesized in our laboratory[11] in 3 steps starting from (R)-2-amino-1-propanol.

RADIOACTIVITY COUNTING

Determination of total radioactivity was carried out on a Packard 2000 CA tri-carb liquid scintillation analyzer, using 20 ml counting vials and Opti-fluor™ Packard liquid scintillator.

MASS SPECTROSCOPY

The mass spectrometer used was a Finnigan-MAT TSQ 70B triple-quadropole instrument equipped with a FAB xenon gun: Ion Tech 8000 V. Current 2 mA. Matrix: Glycerol.

NMR SPECTROSCOPY

^{1}H-NMR spectra were recorded on a Bruker 400 MHz spectrometer, with chemical shifts δ measured in ppm downfield from internal standard Me_4Si (δ = 0 ppm).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC analyses were performed using a Merck HPLC pump L-6200 with a rheodyne injector (20 µl loop) and a Merck UV-detector L-4000 (operating at 274 nm). Separations were accomplished at RT with a C-18 column (250 x 4,6 mm, 5 µm) from Novo Nordisk A/S, using a mixture of water and acetonitrile. The flow rate was 1.0 ml/min. Radioactivity in the column effluent was monitored with a Radiomatic/Canberra Flo-One beta detector A-515, using a 500 µl liquid flow cell. The ratio of column effluent to liquid scintillator (Opti-fluor™, Packard) was 1:2. Data collection was done by Flo-One data software on a PC-80386 computer. Two different analytical HPLC systems were used. System A: Gradient; 90/10 water/acetonitrile → 40/60 water/acetonitrile from 0-15 min. 40/60 water/acetonitrile → 0/100 water/acetonitrile from 15-30 min. System B: Gradient; 30/70 water/acetonitrile → 0/100 water/acetonitrile from 0-20 min.

TLC

TLC was performed on glass plates (5x20 cm) coated with 0.25 mm silica gel 60 F_{254} (Merck). The mobile phase was CH_2Cl_2:EtOH (90:10) (system I) and EtOAc:heptane (50:50) (system II). Radio-TLC analysis were performed using a Bioscan Imaging Scanner System 200-IBM with an Autochanger 1000.

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