

SYNTHESIS OF [5-¹⁴C]-1,1,12,12-TETRAETHOXY-4,9-DIAZADODECANE DINITRATE

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SUMMARY

The reaction of [1-¹⁴C]succinyl chloride with 3,3-diethoxypropylamine (4) afforded N,N'-bis(3,3-diethoxypropyl)[1-¹⁴C]succinic acid diamide (6, 43.9% chemical and radiochemical yield) and N-(3,3-diethoxypropyl)[1-¹⁴C]succinimide (7, 35.8% chemical and 35.5% radiochemical yield). Reduction of 6 using lithium aluminum hydride followed by reaction with concentrated nitric acid yielded [5-¹⁴C]-1,1,12,12-tetraethoxy-4,9-diazadodecane dinitrate (8) in 16% overall radiochemical yield from [1-¹⁴C]succinyl chloride with a specific activity of 55.4 mCi/mmol (2.05 GBq/mmol) and a radiochemical purity of 97.3%.

Key words: 1-¹⁴C-Succinyl chloride, N,N'-bis(3,3-diethoxypropyl)[1-¹⁴C]succinic acid diamide, N-(3,3-diethoxypropyl)[1-¹⁴C]succinimide, [5-¹⁴C]-1,1,12,12-tetraethoxy-4,9-diazadodecane dinitrate.

INTRODUCTION

In the course of investigations in biological and metabolic studies it became necessary to prepare high specific activity [5-¹⁴C]-1,1,12,12-tetraethoxy-4,9-diazadodecane dinitrate (8). Evidence has been presented that 4,9-diazadodecanedialdehyde (1, n=2, R=CHO) may be an enzymatic product derived from spermine (1, n=3, R=NH₂). It has been suggested that the bisaldehyde (1, n=2, R=CHO) may be responsible for the growth-inhibitory and viral-inactivation effects of spermine due to interaction of the bisaldehyde with DNA. It was subsequently reported that this bisaldehyde inhibits the growth of KB (human epidermoid carcinoma) cells *in vitro* without the need for generation of acrolein as the active cytotoxic agent (1). We now report a facile procedure for the synthesis of high specific activity

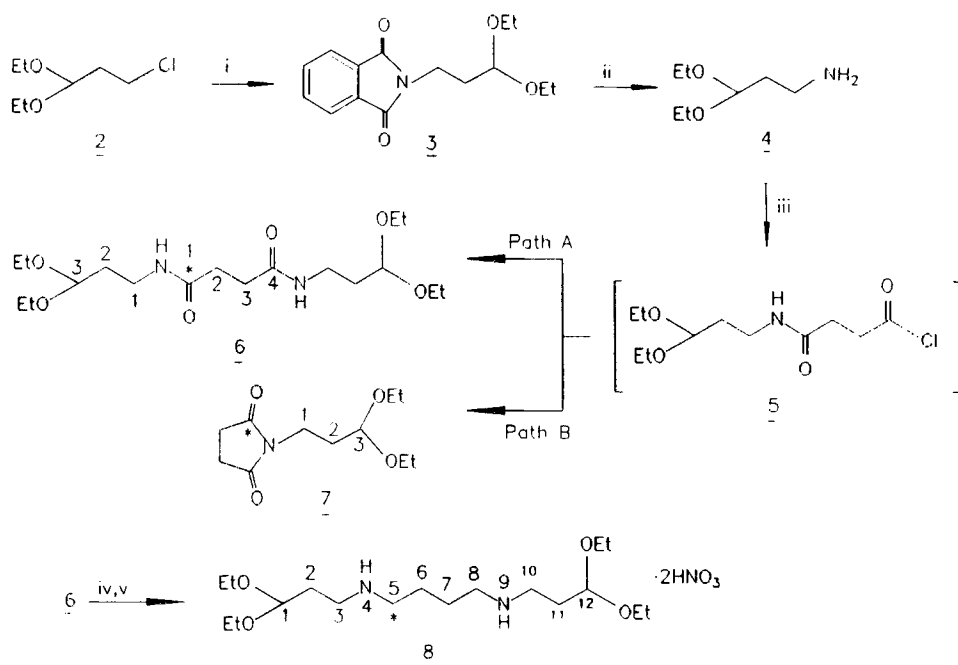
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[5-¹⁴C]-1,1,12,12-tetraethoxy-4,9-diazadodecane dinitrate (**8**) which can be readily converted to the required bisaldehyde analogue. $R-(CH_2)_nNH(CH_2)_4NH(CH_2)_n-R$ (**1**)

RESULTS AND DISCUSSION

In an earlier study an efficient synthetic methodology for the synthesis of high specific activity (55.4 mCi/mmol) [1-¹⁴C]succinyl chloride was developed (**2**). The synthetic methodology illustrated in Scheme I was selected since it was expected to give the target compound **8** having the ¹⁴C-label at the metabolically stable C-5 position. The reaction of [1-¹⁴C]succinyl chloride with 3,3-diethoxypropylamine (**4**), which was synthesized from 3,3-diethoxypropyl chloride (**2**) according to the method of Carroll *et al* (**3**) using the reactions illustrated in Scheme I, yielded a mixture of *N,N'*-bis(3,3-diethoxypropyl)[1-¹⁴C]succinic acid diamide (**6**, 43.9% chemical and radiochemical yield) and *N*-(3,3-diethoxypropyl)[1-¹⁴C]succinimide (**7**, 35.8% chemical and 35.5% radiochemical yield) with a specific activity of 55.4 mCi/mmol, which were readily separated by silica gel column chromatography.

Scheme I



Reagents: *i*, potassium phthalimide, *N*-methylpyrrolidin-2-one, 125°C; *ii*, anhydrous H₂NNH₂, MeOH, reflux; *iii*, [1-¹⁴C]succinyl chloride, CH₂Cl₂, Et₃N; *iv*, LiAlH₄, THF, reflux; *v*, conc. HNO₃, EtOH:Et₂O (2:5 v/v).

The formation of the succinimide (7) indicates that intramolecular cyclization of the intermediate product 5 to yield 7 (Path B) is competitive with further reaction of 5 with 4 (Path A) to afford 6 (see Scheme 1). Reduction of 6 using lithium aluminum hydride and then reaction of the diamine product with concentrated nitric acid yielded [5-¹⁴C]-1,1,12,12-tetraethoxy-4,9-diazadodecane dinitrate (8, 36% chemical and radiochemical yield, specific activity 55.4 mCi/mmol, radiochemical purity 97.3%)

SUMMARY

High specific activity (55.4 mCi/mmol) [5-¹⁴C]-1,1,12,12-tetraethoxy-4,9-diazadodecane dinitrate (8) was synthesized in 16% overall radiochemical yield from [1-¹⁴C]succinyl chloride in a two step reaction sequence.

EXPERIMENTAL

All solvents were glass distilled before use and, where anhydrous solvents were required, these were dried by standard methods. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Whatman MK6F microslides. Silica gel column chromatography was performed using Fisher S704 (60-200 mesh) silica gel. ¹H NMR spectra were acquired using a Bruker AM-300 300 MHz spectrometer. Radioactive counting was performed using Aquasol-2 fluor (New England Nuclear) in a Beckman LS 9000 liquid scintillation counter. The radioactive constituents of reaction mixtures were analyzed by fractionating a developed TLC plate, analyzing the fractions by liquid scintillation counting and plotting histograms of the resulting data (combined TLC-LSC). [1-¹⁴C]Succinyl chloride was prepared using the previously reported procedure (2). 3-Chloropropionaldehyde diethylacetal was purchased from the Aldrich Chemical Company.

N-(3,3-Diethoxypropyl)phthalimide (3)

A mixture of potassium phthalimide (18.5 g, 0.1 mol) and 3-chloropropionaldehyde diethylacetal (2, 16.7 g, 0.1 mol) in *N*-methylpyrrolidin-2-one (200 mL) was allowed to stir for 15 hr at 125°C under an atmosphere of nitrogen. The resulting solution was cooled and poured into water (400 mL). Extraction with ether (4x100 mL), washing the combined ether extracts with water (2x100 mL), drying the ether extract (Na₂SO₄), filtration and removal of the solvent *in vacuo* gave an orange oil.

Purification by elution from a silica gel column (150 g) using dichloromethane: ether (95:5 v/v) as eluent gave **3** as a pale yellow oil (16.5 g, 60%); ^1H NMR (CDCl_3) δ : 1.15 (t, 6H, OCH_2CH_3), 2.03 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}$), 3.52 (m, 2H, OCH_2CH_3), 3.65 (m, 2H, OCH_2CH_3), 3.82 (t, 2H, $\text{NCH}_2\text{CH}_2\text{CH}$), 4.61 (t, 1H, $\text{NCH}_2\text{CH}_2\text{CH}$), 7.75-7.87 (m, 4H, aryl hydrogens). The product **3** was used immediately in the next reaction for the synthesis of **4**.

3,3-Diethoxypropylamine (**4**)

A solution of **3** (25.14 g, 181 mmol) and anhydrous hydrazine (5.81 g, 181 mmol) in HPLC grade methanol (450 mL) was heated at reflux for 4 hr with mechanical stirring. The mixture was cooled to 25°C with continued stirring, filtered and the precipitate was washed with methanol. Removal of the solvent *in vacuo* from the filtrate gave a semi-solid which was stirred with dichloromethane (200 mL) for 5 min at 25°C prior to filtration. Removal of the solvent from the filtrate gave a residue which was distilled under reduced pressure to yield **4** as a colorless liquid (11.0 g, 82.5%); bp 67°C (15 torr), Lit. (1) bp 60-62°C (4 mm); ^1H NMR (CDCl_3) δ : 1.25 (t, 6H, OCH_2CH_3), 1.48 (s, 2H, NH_2 , exchanges with deuterium oxide), 1.84 (m, 2H, $\text{CH}_2\text{CH}_2\text{NH}_2$), 2.84 (t, 2H, $\text{CH}_2\text{CH}_2\text{NH}_2$), 3.58 (m, 2H, OCH_2CH_3), 3.74 (m, 2H, OCH_2CH_3), 4.65 [t, 1H, $\text{CH}(\text{OEt}_2)$].

N,N'-Bis(3,3-diethoxypropyl)[1- ^{14}C]succinic acid diamide (**6**) and *N*-(3,3-Diethoxypropyl)[1- ^{14}C]succinimide (**7**)

A solution of [1- ^{14}C]succinyl chloride (88.5 mg, 0.56 mmol, 31.0 mCi, specific activity 55.4 mCi/mmol) in dichloromethane (5 mL) was added dropwise to a solution of **4** (0.176 g, 1.2 mmol) and triethylamine (0.18 mL, 1.3 mmol) in dichloromethane (5 mL) at -50°C under an atmosphere of nitrogen with stirring. The reaction mixture was then stirred at 25°C for 18 hr. Removal of the solvent *in vacuo* gave a suspension which was filtered. Removal of the solvent from the filtrate gave a residue which was purified by elution from a silica gel column (2.5 x 25 cm) using ethyl acetate:2-propanol (7:1 v/v) as eluant. Fifty fractions of eluant (10 mL each) were collected and the radioactivity present in each fraction was measured by liquid scintillation counting. An activity histogram indicated the

presence of two major products 6 and 7 which were well resolved. Products 6 and 7 exhibited R_f values [Whatman MK6F microslides, ethyl acetate:2-propanol (7:1 v/v)] of 0.24 and 0.73, respectively. Removal of the solvent *in vacuo* from the combined fractions having R_f 0.24 (identical to that of an authentic unlabelled sample) yielded 6 (92.6 mg, 43.9% chemical and radiochemical yield, 13.9 mCi, 0.51 GBq); ¹H NMR (CDCl₃) δ: 1.23 (t, 12H, OCH₂CH₃), 1.84 (m, 4H, NHCH₂CH₂CH), 2.5 (s, 4H, COCH₂CH₂CO), 3.36 (m, 4H, NHCH₂CH₂CH), 3.55 (m, 4H, OCH₂CH₃), 3.7 (m, 4H, OCH₂CH₃), 4.58 (t, 2H, NHCH₂CH₂CH), 6.45 (br s, 2H, NH, exchanges with deuterium oxide). An unlabelled sample of 6 synthesized in the same way exhibited the following mp: 88-90°C [Lit.(4) mp 84-85°C]. Product 6, which was analyzed on a MK6F microslide using the solvent system described above, was divided into 9 equal sections and the silica gel sections were scraped directly into individual scintillation vials for radioactivity measurements. The following results were obtained (section, number of counts, % of total activity applied to the TLC plate): 1, 0, 0.0; 2, 17, 0.06; 3, 17, 0.06; 4, 3, 0.01; 5, 5475, 18.7; 6, 20050, 68.6; 7, 3669, 12.5; 8, 6, 0.02; 9, 0, 0.0. Those sections of the plate corresponding to the R_f for 6 (sections 5-7) accounted for 99.8% of the total activity applied to the TLC plate, *viz* 99.8% radiochemical purity.

Removal of the solvent *in vacuo* from the combined fractions having R_f 0.73 (identical to that of an authentic unlabelled sample) afforded 7 (46 mg, 35.8% chemical and 35.5% radiochemical yield, 11.0 mCi, 0.41 GBq). The product 7, which was analyzed by TLC using the methodology described above for 6 (section, number of counts, % of total activity applied to the TLC plate) indicated the following results for the 9 sections obtained from the TLC plate: 1, 0, 0.0; 2, 10, 0.03; 3, 3478, 12.02; 4, 19850, 68.65; 5, 5522, 19.09; 6, 19, 0.06; 7, 16, 0.05; 8, 18, 0.06; 9, 0, 0.0. Those sections of the plate corresponding to the R_f for 7 (sections 3-5) accounted for 99.7% of the total activity applied to the TLC plate, *viz* 99.7% radiochemical purity. Compound 7 exhibited the following physical and ¹H NMR spectral data: mp 103-105°C; ¹H NMR (CDCl₃) δ: 1.14 (t, 6H, OCH₂CH₃), 1.83 (m, 2H, NCH₂CH₂CH), 2.62 (s, 4H, COCH₂CH₂CO), 3.42 (m, 2H, NCH₂CH₂CH), 3.6 (m, 4H, OCH₂CH₃),

4.48 (t, 1H, $\text{NCH}_2\text{CH}_2\text{CH}$). A sample of unlabelled **7** prepared in the same way exhibited the following spectral data: Exact mass calcd. for $\text{C}_{11}\text{H}_{19}\text{NO}_4$ - Et: 200.0923. Found (high resolution mass spectrum): 200.0923; ^{13}C NMR (CDCl_3) δ : 176.98 ($\text{COCH}_2\text{CH}_2\text{CO}$), 101.14 ($\text{NCH}_2\text{CH}_2\text{CH}$), 61.78 ($\text{O}(\text{CH}_2\text{CH}_3)_2$), 35.06 ($\text{NCH}_2\text{CH}_2\text{CH}$), 31.62 ($\text{NCH}_2\text{CH}_2\text{CH}$), 28.28 and 28.18 ($\text{COCH}_2\text{CH}_2\text{CO}$), 15.30 (OCH_2CH_3)₂.

[5- ^{14}C]-1,1,12,12-Tetraethoxy-4,9-diazadodecane dinitrate (**8**)

A mixture of **6** (85 mg, 0.23 mmol, 12.5 mCi, 55.4 mCi/mmol) and lithium aluminum hydride (19 mg, 0.5 mmol) in dry tetrahydrofuran (20 mL) was heated at reflux for 40 hr under an atmosphere of nitrogen. The reaction mixture was cooled to 25°C, water (0.1 mL) and then sodium hydroxide (0.025 mL of a 15% w/v aqueous solution) was added with stirring. This mixture was stirred for 30 min prior to filtration. Removal of the solvent from the filtrate *in vacuo* gave a residue which was dissolved in ethyl alcohol (0.5 mL), diluted with ether (1.25 mL) and then concentrated nitric acid (0.025 mL) was added dropwise with vigorous stirring. The pale yellow dinitrate salt (**8**) which precipitated was filtered, washed with ether (10 mL) and dried *in vacuo* at 25°C. Compound **8** was obtained as a colorless crystalline solid after dissolution in ethanol:water (20:80 v/v), stirring with charcoal (5 mg) for 5 min, filtration and removal of the solvent *in vacuo*. The product (**8**, 39.3 mg, 4.6 mCi, 36% chemical and 36.8% radiochemical yield, specific activity 55.4 mCi/mmol) exhibited the following physical and spectral data: mp 144-145°C [lit. (4) mp 144-145°C; ^1H NMR (CDCl_3) δ : 1.2 (t, 12H, OCH_2CH_3), 1.77 (m, 4H, H-2, H-11), 2.04 (m, 4H, H-6, H-7), 3.16 (m, 8H, H-3, H-5, H-8, H-10), 3.66 (m, 4H, OCH_2CH_3), 3.78 (m, 4H, OCH_2CH_3), 4.78 (t, 2H, CHOEt).

The radiochemical purity of the dinitrate salt (**8**) was determined after conversion to the free base of **8**. The pH of an aqueous solution of the dinitrate salt (**8**) was adjusted to 11 using aqueous sodium hydroxide to give the free base which exhibited a single spot having R_f 0.26 on a Whatman MK6F microslide using chloroform:methanol:water (60:35:5 v/v/v) as development solvent. The free base of **8**, which was analyzed by TLC using the methodology

described previously for 6 (section, number of counts, % of total activity applied to the TLC plate) using the solvent system described above, indicated the following results for the 18 sections obtained from the TLC plate: 1, 12, 0.05; 2, 9, 0.04; 3, 4, 0.02; 4, 27, 0.12; 5, 77, 0.34; 6, 330, 1.48; 7, 1848, 8.32; 9, 8119, 36.5; 10, 956, 4.3; 11, 247, 1.1; 12, 173, 0.77; 13, 130, 0.58; 14, 127, 0.57; 15, 112, 0.50; 16, 91, 0.40; 17, 16, 0.07; 18, 0, 0.00. Those sections of the plate corresponding to the R_f for the free base of 8 (sections 6-12) accounted for 97.3% of the total activity applied to the plate, viz 97.3% radiochemical purity.

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