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SYNTHESIS OF TRITIUM LABELLED SPARSOMYCIN

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SUMMARY

The synthesis is described of tritiated sparsomycin (5), a broad-spectrum antitumor antibiotic, with high specific activity (31.4 Ci/mmol, 1.16 TBq/mmol). The radiolabel was introduced by reduction of the cysteine methyl ester 1 with lithium borotritide. ^{3}H NMR analysis of the final product showed the concomitant formation of the mono- and ditritiated compound in a ratio of 2:1.

Keywords: tritiation, protein synthesis inhibitor, antitumor antibiotic

INTRODUCTION

Sparsomycin and several more potent analogues are broad-spectrum antibiotics.[1-3] These drugs act on the ribosomal level and affect protein biosynthesis. Currently, sparsomycin and some selected derivatives are evaluated in preclinical investigations for their use as anticancer agents. For in vitro drug-receptor and in vivo pharmacokinetic studies we required a radiolabelled drug. We recovered from the literature two references that describe biochemical studies using "tritiated" sparsomycin derivatives.[4,5] However, no information was reported on their synthesis, purity and structure identification. This report describes our approach to the chemical synthesis and characterization of optically active [^{3}H]sparsomycin (5).

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RESULTS AND DISCUSSION

[^{3}H]Sparsomycin (5) was prepared in the following way. The cysteine methyl ester 1 was reduced with lithium borotritide, prepared in situ from NaB^3H_4 (s.a. 64.9 Ci/mmol, 2.4 TBq/mmol) and LiI (see Scheme I).[6] Subsequently, 2 was reacted with sodium methyl mercaptide to give compound 3.[6] The amino protecting group of 3 was removed by treatment with neat trifluoroacetic acid,[6] and the resulting amine was coupled subsequently with the pentafluorophenyl ester 4 to yield 1.4 mCi of the desired end product 5. The chemical and radiochemical purity of 5 was checked by HPTLC (silica gel, eluent MeOH/CH_2Cl_2 2:8) and by HPLC (μBondapak C8, eluent MeOH/H_2O 1:9). The specific activity of 31.4 Ci/mmol (1.16 TBq/mmol) of [^{3}H]sparsomycin was determined from a UV calibration line constructed for known amounts of cold sparsomycin, by HPLC analysis (UV detection at 302 nm)/liquid scintillation counting. The calculated specific activity is in good agreement with the isotopic abundance of about 60% of the sodium tritide used.

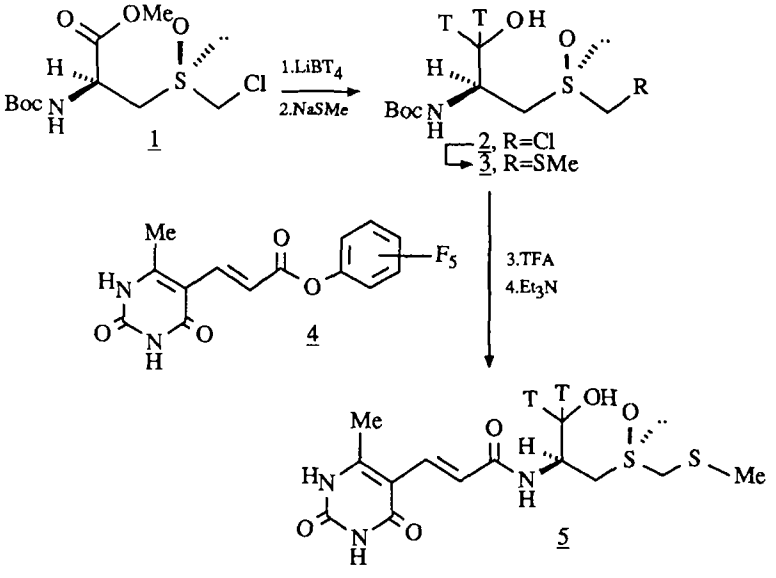
A tritium NMR spectrum was run of the intermediate 2 and of the final product 5 for the determination of the position and distribution of the tritium in the labelled drug. This technique has proven to be a very powerful one in the analysis of tritiated drugs.[7-9] Broad-band ^{1}H decoupled ^{3}H NMR analysis of 2 showed that this product is a mixture of the mono- and ditritiated compound; signals at 3.485, 3.518, 3.537 (two overlapping signals), 3.572 and 3.581 ppm were observed (see Figure 1a). By introduction of one tritium atom in the molecule two diastereomers are formed; the singlets at 3.537 and 3.581 represent these diastereoisomers. The signals at 3.485, 3.518, 3.537 and 3.572 form an AB pattern that results from the two diastereotopic tritium atoms in the ditritiated compound. That this AB signal is shifted upfield in comparison with the signals of the monotritiated diastereomers, is in agreement with the tritium isotope effect.[10,11] Integration of the NMR signals shows a ratio mono-/di- tritiated compound of approximately 2:1.

[^{3}H]Sparsomycin 5 shows singlets at 3.646, 3,668 and 3.684 ppm in the tritium NMR spectrum (see Figure 1b). The presence of two diastereomers is deduced by the presence of two singlets seen at 3.668 and 3.684 ppm. The broadened singlet at 3.646 ppm originates from the AB pattern that is expected for the ditritiated compound (vide supra). This simplified AB pattern again is situated at higher field than the signals of both monotritiated compounds (vide supra). Integration of the NMR signals confirms the ratio of the mono- and ditritiated compound to be approximately 2:1 (vide supra).

In pilot studies we had prepared tritiated sparsomycin with a specific activity of 0.3 Ci/mmol (11 GBq/mmol), using commercially available sodium borotritide. We observed that the yield of

the metal hydride reduction in this case was far better than in case of the synthesis of tritiated sparsomycin of high specific activity. Tritium NMR analysis of the product with low specific activity, i.e. 0.3 Ci/mmol (11 GBq/mmol), revealed only the two singlets of the two diastereomeric monotritiated compounds of equal intensity (see Figure 1c).

Scheme I



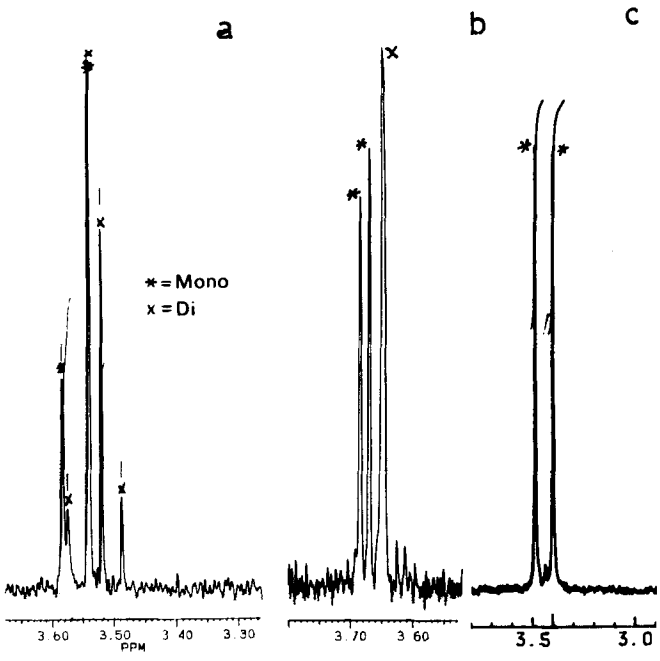
In conclusion, we have been able to synthesize tritium labelled sparsomycin in high specific activity by metal hydride reduction of a suitable precursor. Tritium NMR analysis of the final product pointed to a ratio of mono- over ditritiated compound of approximately 2:1. Investigations on the interaction with the peptidyl transferase and on the pharmacokinetic behaviour in mice of [^{3}H]sparsomycin (5) are in progress.

EXPERIMENTAL SECTION

The tritiation reaction was carried out at Amersham International plc, Cardiff, U.K. by their tritium labelling service. ^{3}H NMR spectra recorded with broad-band ^{1}H decoupling, were measured on a Bruker AM360 spectrometer operating at 384,138 MHz.[7-11] Chemical shifts (δ) are referred to "ghost" - Me_4Si, the value of which was obtained by multiplying the ^{1}H frequency of Me_4Si by 1,06663975. For HPTLC an Isomess IM-3016 Radio-TLC-Analyser was used. HPLC analyses

were obtained on a Waters 6000A system equipped with a Pye Unicam 4025 UV spectrophotometer and a Berthold LB503 liquid scintillation counter.

Figure 1. Tritium NMR spectra of compounds **2** (a) and **5** (b and c)



ScRs [1-^{3}H]-2-[N-(tert-Butyloxycarbonyl)amino]-3-[(Chloromethyl)sulfinyl]-propanol (2). To a solution of 1 Ci (37 GBq) of sodium borotritide (Amersham, s.a. 64.9 Ci/mmol, 2.4 TBq/mmol) in 0.5 ml of dry THF was added subsequently 10 mg of LiI and 20 mg of 1 at -70 °C.[6] The reaction mixture was stirred for 20 h at ambient temperature in a nitrogen atmosphere. The excess $LiBT_4$ was destroyed with a few drops of concentrated HOAc. After neutralization with Na_2CO_3, the reaction mixture was concentrated, the remainder was dissolved in $MeOH/CH_2Cl_2$ (95:5), the solution was filtrated and the solvents again were evaporated with a stream of nitrogen in order to remove any labile tritium. The crude product (263 mCi, 9.7 GBq) was purified on silica gel (eluent CH_2Cl_2/MeOH, 95:5) giving 24.6 mCi (910 MBq) of 2. The product co-migrated with unlabeled 2 on TLC. ^{3}H NMR (CD_3OD) δ 3.48593 (s, CT_2OH), 3.518 (s, CT_2OH), 3.537 (2x s, CT_2OH and CH*T*OH), 3.572 (s, CT_2OH), 3.581 (s, CH*T*OH).

ScRs [1-^{3}H]-2-[N-(tert-Butyloxycarbonyl)amino]-3-[(methylthio)methylsulfinyl]-propanol (3). Product 2 was dissolved in 1 ml of oxygen-free EtOH and 5 mg of NaSMe were added.[6] After

stirring for 20 h at room temperature in a nitrogen atmosphere, the solvent was evaporated with a stream of nitrogen and the residue was purified on silica gel (eluent $CHCl_3$/MeOH, 93:7) to yield 20 mCi (740 MBq) of pure 3. The product had the same Rf value as the unlabeled material.

ScRs [1-^{3}H]-2-[(E)-β-(6-methyl-5-uracilyl)acrylamido]-3-[(methylthio)methylsulfinyl]-propanol (5). Product 3 was stirred in 2 ml of neat TFA for 45 min at ambient temperature.[6] The amino deprotection reaction was followed on TLC (eluent $CHCl_3$/MeOH, 8:2). After removal of the excess TFA, the residue was dissolved in 2 ml of DMF. The solution was neutralized with Et_3N and subsequently, 25 µl of Et_3N and 10 mg of the pentafluorophenyl ester 4[12] were added. The reaction mixture was stirred for 20 h at room temperature and the solution was then lyophilized. The product was first purified by chromatography on silica gel (eluent $CHCl_3$/MeOH, 9:1) and secondly by chromatography on HPLC (Supelco RP8, eluent MeOH/H_2O, 1:9) yielding 1.4 mCi (52 MBq) of the pure compound (>96% by HPLC) next to 0.7 mCi (26 MBq) of an impure fraction of the desired compound. ^{3}H NMR (CD_3OD) δ 3.646 (s, CT_2OH), 3.668 and 3.684 (2x s, 2x CHTOH).

[^{3}H]sparsomycin (0.3 Ci/mmol, 11 GBq/mmol): ^{3}H NMR (DMSO-d6) δ 3.402 and 3.668 (2x s, 2x CHTOH).

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