THE SYNTHESIS OF [^{14}C] and [$^{3}H_2$] SK&F L-94901.

A NOVEL THYROMIMETIC

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SUMMARY

A novel thyromimetic SK&F L-94901, L-3,5-dibromo-3'-(6-oxo-1,6-dihydro pyridazin-3-ylmethyl)thyronine, has been labelled with ^{14}C and ^{3}H for drug metabolism and nuclear binding studies. A six stage synthesis from [ß-^{14}C]tyrosine is described. The overall radiochemical yield was 13.7%.
Syntheses of [$^{3}H_2$]SK&F L-94901 of 1.22Ci mmol^{-1} and 13.9Ci mmol^{-1} are described, and the instability of these compounds noted.

KEYWORDS: autoradiolysis, bromination, h.p.l.c., iodonium salt, L-[2,6-$^{3}H_2$]tyrosine, thyromimetic, L-[ß-^{14}C]tyrosine.

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INTRODUCTION

Hypercholesterolaemia has been long recognised as a significant risk factor in atherosclerosis. There is considerable evidence linking hypercholesterolaemia with coronary heart disease.[1] Thyroid hormones have been used to reduce plasma cholesterol concentrations in atherosclerotic patients[2], however, their use is limited due to adverse cardiac effects.[3] A selective thyromimetic[4], free from these adverse effects would be a therapeutically useful hypocholesterolaemic agent. SK&F L-94901[5] is a selective thyromimetic under investigation in our

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laboratories for the treatment of hypercholesterolaemia. We report here the syntheses of [^{14}C]SK&F L-94901 and [$^{3}H_2$]SK&F L-94901 which were required for drug metabolism and nuclear binding studies.

DISCUSSION

1. [^{14}C]SK&F L-94901

The synthesis of [^{14}C]SK&F L-94901(7*)[5] is illustrated in Scheme 1. Treatment of L-[ß-^{14}C]tyrosine[6,7] in glacial acetic acid with two equivalents of bromine gave, smoothly and in high yield, L-3,5-dibromo[ß-^{14}C]tyrosine (2*). To ensure that the key step in this synthesis, the coupling of the tyrosine entity to the iodonium salt[8,9] (5) proceeded cleanly, selective protection of the acid and amine functionalities of the 3,5-dibromotyrosine was necessary. This was readily achieved in two steps. Firstly, the methyl ester was prepared, under standard conditions, in virtually quantitative yield. This was, in turn, reacted with trifluoroacetic anhydride in chloroform/ethyl acetate (1:1) to give, on aqueous work up, the [^{14}C] diprotected derivative (4*) in 66% radiochemical yield from L-[ß-^{14}C]tyrosine.

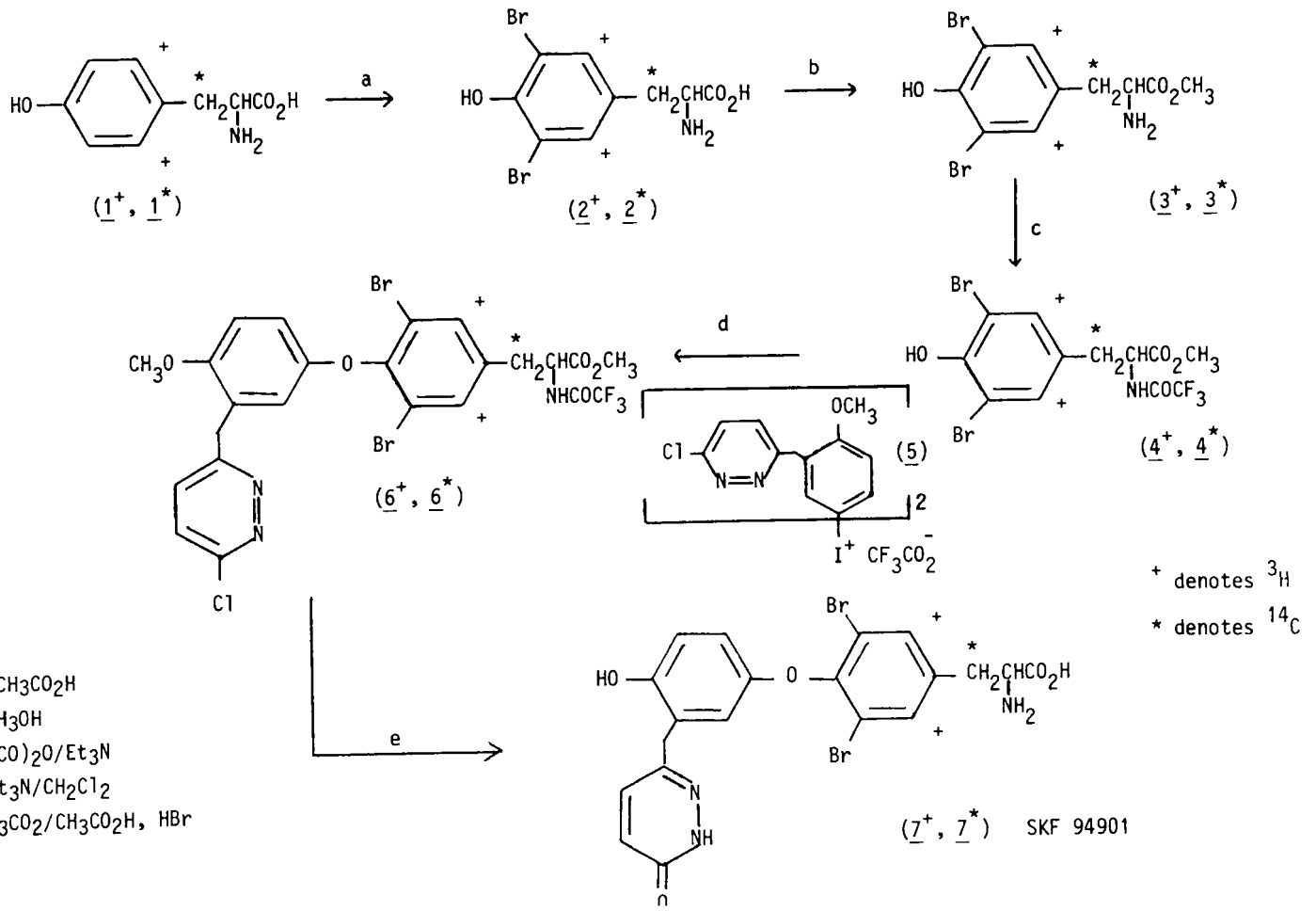
The molecular framework was now completed by coupling of the [^{14}C] diprotected tyrosine (4*) with the iodonium salt (5) by stirring at room temperature, in dichloromethane, in the presence of copper-bronze and triethylamine. Purification of this crude material by column chromatography gave the [^{14}C] triprotected intermediate (6*) in 60% radiochemical yield.

Deprotection to give [^{14}C]SK&F L-94901 was now achieved in two steps. Treatment with sodium acetate in glacial acetic acid gave the [^{14}C]pyridazinone which, on subsequent reaction with hydrobromic acid in glacial acetic acid yielded the crude product. This was purified by semi-preparative h.p.l.c. to give [^{14}C]SK&F L-94901 (7*) (radiochemical purity >97.6% by t.l.c.) in overall radiochemical yield of 13.7% from L-[ß-^{14}C]tyrosine. This material contained 2.5% of the corresponding D-(+)-isomer, as determined by h.p.l.c. analysis of the (+) phenylethyl isocyanate derivative.[10]

2. [$^{3}H_2$]SK&F L-94901 - Low Specific Activity Synthesis

With a robust synthesis of [^{14}C]SK&F L-94901 in hand, attention was

Scheme 1


(1+, 1*)
(2+, 2*)
(3+, 3*)
(4+, 4*)
(5)
(6+, 6*)
(7+, 7*) SKF 94901
a) Br2/CH3CO2H
b) H+/CH3OH
c) (CF3CO)2O/Et3N
d) Cu/Et3N/CH2Cl2
e) NaCH3CO2/CH3CO2H, HBr
+ denotes 3H
* denotes 14C

now directed toward the preparation of [$^3H_2$]SK&F L-94901 (see Scheme 1). We required ~15mCi at 10Cimmol^{-1} which represents ~800μg of [$^3H_2$]SK&F L-94901 ($\underline{7}^+$).

Initially, we proceeded with a trial low specific synthesis, commencing with L-[2,6-$^3H_2$]tyrosine[11] (5mCi), of specific activity, 1.4Cimmol^{-1} (~650μg). The route utilised for the synthesis of [^{14}C]SK&F L-94901 was followed, incorporating modifications of work up procedures etc, appropriate to a microgram scale preparation (see experimental section for full details), giving crude [$^3H_2$]SK&F L-94901 (1.29mCi). This crude material was readily purified on an analytical t.l.c. plate (Analtech GF_{254} Silica, ethyl acetate/methanol/conc. ammonium hydroxide 5:1:1, by vol). In all, 540μCi, 10.8% radiochemical yield from L-[2,6-$^3H_2$]tyrosine, of [$^3H_2$]SK&F L-94901 of radiochemical purity >97% by t.l.c., were isolated. The specific activity, as determined by an h.p.l.c. peak height analysis[12], was 1.22Cimmol^{-1} indicating that ~13% H-T exchange occurred during the synthesis.

3. [$^3H_2$]SK&F 94901 - High Specific Activity Synthesis

L-[2,6-$^3H_2$]tyrosine[11] (250mCi, 39Ci mmol) was smoothly brominated yielding L-3,5-dibromo[2,6-$^3H_2$]tyrosine in 93.2% (232.9mCi) crude radiochemical yield. This crude material was dissolved in methanol saturated with HCl gas and stirred at room temperature for 3h when t.l.c. indicated the reaction had gone to completion. Aqueous work up gave the crude [$^3H_2$]-methyl ester ($\underline{3}^+$) in only 42% radiochemical yield (98.0mCi). (An 80% yield was achieved for this esterification during the low specific activity synthesis described above. This unexpectedly low radiochemical yield was attributed to extensive H-T exchange, possibly acid catalysed).

The remaining steps in the synthesis proceeded smoothly to yield crude [$^3H_2$]SK&F 94901 ($\underline{7}^+$) (35mCi, 14% radiochemical yield from L-[2,6-$^3H_2$]tyrosine) and of ~80% radiochemical purity as assessed by t.l.c. (ethyl acetate/methanol/conc. ammonia 5:1:1 by vol).

However, purification of this material proved extremely difficult. The thin layer chromatography method developed for the low specific activity synthesis, gave material of <90% radiochemical purity. Two-dimensional chromatography showed that significant decomposition

was not occurring during development. An h.p.l.c. purification method was consequently developed. Samples (~100µg injections) were purified on an analytical h.p.l.c. column [µ-Bondapak-C18, eluted with $CH_3CN/0.07MPO_4^{3-}$, pH = 2.2 (1:4v/v)]. The radiochemical purity of [$^{3}H_2$]SK&F L-94901, immediately after purification, was >98.0% as determined by t.l.c. The specific activity, as determined previously[12] was 13.9Cimmol^{-1}. As the specific activity of the starting L-[2,6-$^{3}H_2$]tyrosine was 39Cimmol^{-1}, approximately 64% H-T exchange occurred during this synthesis.

This high specific activity [$^{3}H_2$]SK&F L-94901 proved extremely unstable, decomposing at a rate of ~1-2% per day over a period of two weeks, as shown by t.l.c. analysis. (It should be noted that the [$^{3}H_2$]SK&F L-94901 of specific activity 1.22Ci mmol^{-1} previously prepared, was also of low stability, decomposing at ~1-2% per week). Consequently this high specific activity material was purified, analysed and supplied to the Pharmacology Department, for use in nuclear binding studies, all on the same day. In all 2.072mCi, of [^{3}H]SK&F L-94901 of specific activity 13.9Cimmol^{-1} were prepared in this manner.

EXPERIMENTAL

L-[ß-^{14}C]Tyrosine[6] was obtained from ICI Physics and Radioisotope Services, Billingham, and L-[2,6-$^{3}H_2$]tyrosine[11] from Amersham International plc. Semi-preparative h.p.l.c. purification was carried out on an Arcksil 10µ C18 column (250mm x 22mm i.d.) with a Gilson preparative system. Radiochemical purities were determined on Analtech 02511 silica gel plates utilising a Berthold LB2832 Automatic Linear Analyser.

L-3,5-Dibromo[ß-^{14}C]tyrosine (2*)

L-[ß-^{14}C]Tyrosine (85mCi, 53.5mCi mmol^{-1}, 1.59mmol, 288mg) was dissolved in glacial acetic acid (5ml) and bromine (163µl, 3.16mmol) added. The mixture was stirred at 80°C for 2h, when t.l.c. (n-butanol/acetic acid/water 12:3:5 by vol., visualised with ninhydrin) showed the reaction had gone to completion. The solvent was removed under vacuo and the residue slurried in water, the pH was adjusted to 6 with saturated aqueous sodium bicarbonate and the L-3,5-dibromo[ß-^{14}C]-tyrosine (515mg, 1.51mmol, 95.0%) (2*) filtered off as a colourless solid.

Methyl L-3,5-dibromo[ß-^{14}C]tyrosinate (3*)

L-3,5-Dibromo[ß-^{14}C]tyrosine (515mg,1.51mmol) (2*) was dissolved in methanol (10ml) and dry HCl gas bubbled slowly through for 5h, when t.l.c. (ethyl acetate/methanol/ammonia 5:1:1 by vol.) showed the reaction had gone to completion. The solvent was evaporated under vacuo and the residue dissolved in water and the pH adjusted to 6 with saturated aqueous sodium bicarbonate. The precipitated ester (3*) was filtered off and dried under vacuum (536mg, 1.51mmol, 100%).

Methyl L-3,5-dibromo-N-trifluoroacetyl[ß-^{14}C]tyrosinate (4*)

To a stirred suspension of the [^{14}C] methyl ester (3*) prepared above, in chloroform/ethyl acetate (1:1 v/v) (10ml) was added trifluoroacetic anhydride (TFAA) (337µl, 2.39mmol), and the mixture stirred at room temperature for 3h, when a further aliquot of TFAA (225µl, 1.60mmol) was added. After 5h total reaction time, an addition aliquot (150µl, 1.06mmol) of TFAA was added the reaction continued for 18h. Water (10ml) was added, and the mixture stirred for 30 min. The pH of the aqueous phase was adjusted to 6 with saturated aqueous sodium bicarbonate, the layers separated and the organic extract dried over $MgSO_4$. On filtration and evaporation, of the organic solvent the required methyl L-3,5-dibromo-N-trifluoroacetyl [ß-^{14}C]tyrosinate (4*) (565.6mg 1.25mmol, 56.0mCi, 66% radiochemical yield from L-[ß-^{14}C]tyrosine) was obtained.

Methyl L-3,5-dibromo-3'-(6-chloropyridazin-3-ylmethyl)N-trifluro-acetyl [ß-^{14}C]thyroninate (6*)

To a solution of methyl L-3,5-dibromotyrosine-N-trifluoroacetyl [ß-^{14}C]tyrosinate (565.6mg, 1.25mmol) (4*) in dichloromethane (8ml), containing triethylamine (240µl, 1.73mmol), was added copper bronze (65mg), and bis [3-(6-chloropyridazin-3-ylmethyl)-4-methoxyphenyl] iodonium trifluoroacetate (5) (1.485g, 2.10mmol) and the mixture stirred at room temperature for 18h., when t.l.c. (dichloromethane/methyl ethyl ketone 95.5v/v) showed the reaction had gone to completion. The reaction mixture was filtered through a short column of silica gel to remove the inorganics, and the crude (6*) (1.52g) purified by column chromatography (silica gel, 70-230 mesh 25g, eluted with dichloromethane/methylethyl ketone 96:4 v/v), to give the required [^{14}C] triprotected SK&F L-94901 (6*) (510mg, 0.75mmol, 33.9mCi, 60% radiochemical yield).

[^{14}C]SK&F L-94901, L-3,5-dibromo-3'-(6-oxo-1,6-dihydropyridazin-3-ylmethyl [ß-^{14}C]thyronine (7*)

The [^{14}C] triprotected derivative (6*) (510mg, 0.75mmol), prepared above was dissolved in acetic acid (5ml), and sodium acetate (104mg, 1.27mmol) added. The mixture was heated under reflux for 3h., and the solvent removed under vacuum. To the residue (446mg) was added 40% aqueous HBr (1.5ml) and glacial acetic acid (1.5ml) and the mixture heated under reflux for 18h. The solvent was evaporated to dryness under vacuum, water was added to the residue, and the pH adjusted to 6. The precipitated crude [^{14}C]SK&F L-94901 was filtered off and dried (305mg). This was dissolved in 0.1M sodium hydroxide solution and purified by semi-preparative h.p.l.c. (5--> 40% acetonitrile in water over 20 min, 15mlmin^{-1}, uv 295nm). The appropriate fractions were combined, the solvent removed under vacuum and the residue dissolved in 0.1M sodium hydroxide solution. The pH was adjusted to 6 with 0.1M hydrochloric acid and the precipitated [^{14}C]SK&F L-94901 filtered off and dried under high vacuum (115mg, 0.21 mmol, 101µCi mg^{-1}, 11.615mCi), in overall radiochemical yield of 13.7% from L-[ß-^{14}C]tyrosine. The radiochemical purity of this material was determined by t.l.c. in three systems, i) n-butanol/pyridine/water (1:1:1, by volume), 98.1%, ii) chloroform/methanol/acetic acid (20:10:1, by volume), 97.6% and iii) ethyl acetate/methanol/conc. ammonium hydroxide (5:1:1, by volume), 99.6%. The enantiomeric excess[10] was 95.0%, as determined by h.p.l.c. analysis of the (+)-phenyl ethyl isocyanate derivative.

L-3,5-Dibromo[2,6-$^{3}H_2$]Tyrosine (2^{+})

L-[2,6-$^{3}H_2$]Tyrosine (250mCi, 39Cimmol^{-1}, 1.18mg, 0.0064mmol) was dissolved in glacial acetic acid (100µl) containing bromine (0.659µl, 0.0128mmol) in a 5ml round bottom flask, and heated at 80°C for 1.5h, when a further 100µl of glacial acetic acid were added. Following a further 1h reaction, bromine (10µl of a 6.57µl solution in 100µl of acetic acid) were added, and reaction continued for another 6h. The cooled solution was evaporated to dryness. T.l.c. (n-butanol/acetic acid/water 12:3:5, by vol) showed ~40% reaction. The residue was dissolved in the bromine/acetic acid solution (100µl) and transferred to a reacti-vial^{R} with acetic acid washings. The vial was sealed and the contents heated at 80°C for a further 3h. On cooling the solvent was evaporated to dryness. T.l.c. of the residue (system as above) showed no remaining L-[2,6-$^{3}H_2$]tyrosine and the required product to be ~80% radiochemically pure (232.9mCi, 93.2% yield).

Methyl L-3,5-dibromo[2,6-$^3H_2$]tyrosinate (3^+)

To L-3,5-dibromo[2,6-$^3H_2$]tyrosine (2^+) (232.9mCi) was added methanol saturated with HCl gas (~3ml), and the mixture stirred at room temperature for 3h, when t.l.c. (ethyl acetate/methanol/ammonia 5:1:1, by vol) showed the reaction had gone to completion. The solvent was removed under vacuum and residue dissolved in water (1ml). The pH was adjusted to 6 with sodium bicarbonate and the solution extracted (x3) with ethyl acetate (2ml). The combined organics were evaporated and the residual methyl L-3,5-dibromo[2,6-$^3H_2$]tyrosinate (3^+) (98.0mCi, 42.2% yield) dried under high vacuum. The aqueous layer contained ~13mCi.

Methyl L-3,5-dibromo-N-trifluoroacetyl-[2,6-$^3H_2$]tyrosinate (4^+)

Methyl-L-3,5-dibromo[2,6-$^3H_2$]tyrosinate (3^+) (98.0mCi) was dissolved in ethyl acetate (200µl) and trifluroacetic anhydride (10µl) and triethylamine (15µl) were added. After 4h. at room temperature the solvent was removed under vacuum. The residue was partitioned between ethyl acetate/chloroform 1:1, v/v (1ml) and water (1ml). The pH of the aqueous layer was adjusted to 6 with saturated aqueous sodium bicarbonate. The layers were separated and the aqueous phase extracted (x1) with ethyl acetate (2ml). The combined organics were evaporated, and the product, methyl L-3,5-dibromo-N- trifluoroacetyl[2,6-$^3H_2$]-tyrosinate (4^+) (98mCi, 100%) dried under high vacuum.

Methyl L-3,5-dibromo-3'-(6-chloropyridazin-3-ylmethyl)-N-trifluoroacetyl [2,6-$^3H_2$]thyroninate (6^+)

To a stirred solution of (4^+) (98mCi, nominally 0.0064mmol) in dichloromethane (200µl) in a reacti-vial^R was added triethylamine (2µl), bis [3-(6-chloropyridazin-3-ylmethyl)-4-methoxyphenyl]iodonium trifluoracetate (5) (9mg, 0.0128mmol) and finely ground copper bronze (5mg). After 18h stirring at room temperature t.l.c.(dichloromethane/ methylethyl ketone 95:5, v/v) showed little reaction.

Triethylamine (2µl) copper bronze (5mg) and the iodonium salt (5) (9mg) were then added and reaction continued for 6h. The reaction mixture was applied to an analytical t.l.c. plate (Analtech 02511 silica, 0.25mm, 5 x 20cm) and developed in dichloromethane/methyl ethyl ketone (95:5, v/v).

The appropriate band was removed and the methyl L-3,5-dibromo-3'-(6-chloropyridazin-3-ylmethyl)-N-trifluoracetyl [2,6-$^{3}H_2$]thyroninate ($\underline{6}^{+}$) (45.0mCi, 95% radiochemically pure by t.l.c.) eluted with dichloromethane/ether (4:1, v/v).

[$^{3}H_2$]SK&F L-94901, L-3,5-Dibromo-3'-(6-oxo-3(1H)pyridazin-3-ylmethyl)[2,6-$^{3}H_2$]thyronine ($\underline{7}^{+}$)

The protected derivative ($\underline{6}^{+}$) (45.0mCi, nominally 0.0029mmol) was dissolved in glacial acetic acid (75µl) and sodium acetate (470µg, 0.0057mmol) added, and the mixture heated at reflux for 4h. Aqueous hydrobromic acid (40%, 50µl) was now added and reaction continued for a further 5h, when t.l.c. (ethyl acetate/methanol/ammonia 5:1:1, by vol) indicated complete conversion to [$^{3}H_2$]SK&F L-94901. (35mCi, 80% radiochemical purity).

Several attempts were made to purify this material by thin layer chromatography (Analtech 02511 silica, 0.25mm, 5 x 25cm plate; ethyl acetate/methanol/ammonia 5:1:1, by vol) but [$^{3}H_2$]SK&F L-94901 could never be isolated in greater than 90% radiochemical purity by this method (see earlier discussion).

Purification was achieved by reverse phase h.p.l.c. (µ-Bondapak C18 4.6mm i.d. x 30cm, eluted with CH_3CN/0.07M PO_4^{3-} pH = 2.2, 1.5ml min^{-1} UV detection at 295nm, ~100µg injections). A sample immediately following purification had a radiochemical purity by t.l.c. (ethyl acetate/ methanol/ammonia, 5:1:1 by vol) of >98.0%. However, following storage at -20°C for 5 days, the radiochemical purity had dropped to 89.8%.

In all 2.072mCi of [$^{3}H_2$]SK&F L-94901 of specific activity 13.9Cimmol^{-1}, determined by h.p.l.c. peak height analysis[12], and radiochemical purity >98.0% (by t.l.c.) were purified, isolated, analysed and supplied for use on the same day.

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6. Supplied by I.C.I., Billingham. No D-(+)-tyrosine was detectable by h.p.l.c. following treatment of resolved tyrosine with L-(-)-tyrosine decarboxylase.

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10. Novapak C18, eluted with CH_3CN 0.07M PO_4^{3-} (pH = 2.2) 30:70 v/v at $1ml min^{-1}$, uv detection at 295nm.

11. Supplied by Amersham International p.l.c. This material contained 3.8% D-tyrosine, determined by use of D-amino acid oxidase.

12. μ-Bondapak-C18 eluted with CH_3CN/0.07MPO_4^{3-}, pH = 2.2 (3:7 v/v) at $1ml min^{-1}$, u.v. detection at 295nm.