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RADIOLABELLED AMINO ACIDS: SYNTHESIS OF N^G-MESITYLENESULFONYL-N^2-[^{3}H]METHYL-ARGININE

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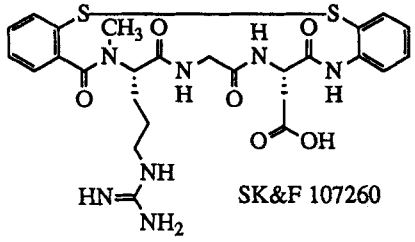
Summary

The N^2-methyl amino acid N^G-mesitylenesulfonyl-[^{3}H]methylarginine has been prepared in high specific activity tritium labelled form by treating methyl N^2-2,4-dimethoxybenzyl-N^G-mesitylenesulfonyl-arginate with [^{3}H]methyl iodide and silver(I) oxide. Removal of the dimethoxybenzyl group by transfer hydrogenation (4.4% formic acid in methanol/Pd black) followed by saponification gave N^G-mesitylenesulfonyl-[^{3}H]methylarginine in 30-38% overall from [^{3}H]methyl iodide. This amino acid was ultimately used in the preparation of tritium labelled SK&F 107260 which is a cyclic peptide showing fibrinogen receptor antagonism.

Key Words: Arginine, N-Methylation, Methyl Iodide

Introduction

SK&F 107260 is a fibrinogen receptor antagonist being examined as a potential treatment for platelet-dependent cardiovascular, cerebrovascular and peripheral vascular diseases (1, 2). Tritium labelled SK&F 107260 at high specific activity (80-90 Ci/mmol) was required for receptor binding assays in the screening of various fibrinogen receptor antagonists.



SK&F 107260

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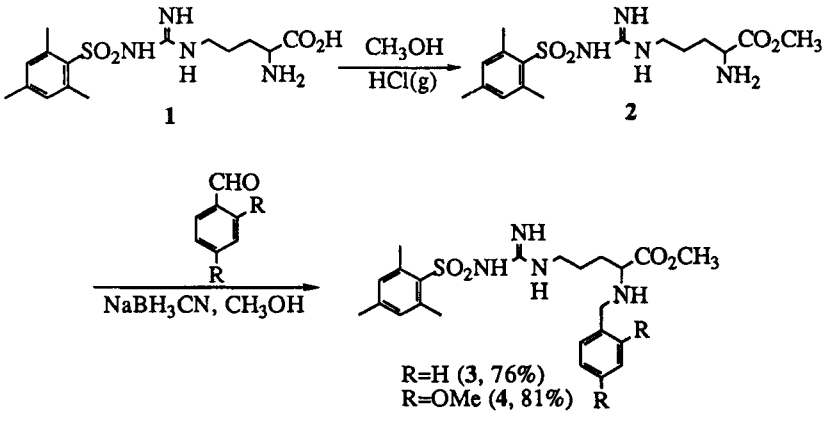
The high specific activity that was required necessitated the incorporation of at least three tritium atoms into the peptide. Labelling the N-methyl group in the L-arginine residue offered the most direct route to preparing high specific activity tritium labelled SK&F 107260. The labelled arginine could then be incorporated into SK&F 107260 via solution phase peptide synthesis.

Discussion

Route Development: Synthesis of N-methyl amino acids is usually accomplished by reductive methylation with formaldehyde on an N-benzyl amino acid followed by removal of the benzyl group (3, 4). Such a procedure is not suitable for the preparation of high specific activity tritium labelled N^2-[^{3}H]methyl amino acids. High yield N-methylation of alanine, phenylalanine, valine and isoleucine has been achieved by treating a protected amino acid with methyl iodide-silver(I) oxide (5). This procedure, if it could be extended to the methylation of arginine using [^{3}H]methyl iodide, would offer a convenient route to tritiated arginine.

A precursor suitable for N-methylation was prepared as shown in Scheme I. N^G-mesitylenesulfonyl-arginine (**1**) was converted to its methyl ester (**2**) which then was reductively alkylated by treatment with either benzaldehyde or 2,4-dimethoxybenzaldehyde

Scheme I



and sodium cyanoborohydride (6) to provide an amino acid (**3** or **4**) suitable for N-methylation. Trial N-methylations showed that arginine could indeed be efficiently methylated, even with methyl iodide as the limiting reagent. The best methylation results (Table 1) were obtained when the alpha nitrogen was activated with a 2,4-dimethoxybenzyl group. The N-benzyl arginine was of intermediate activity and gave moderate yields of the N-methylated amino acid. The BOC derivative, with a deactivated alpha nitrogen, gave no methylation product.

Table 1

N-Methylation of Arginine with Methyl Iodide/Silver(I) Oxide

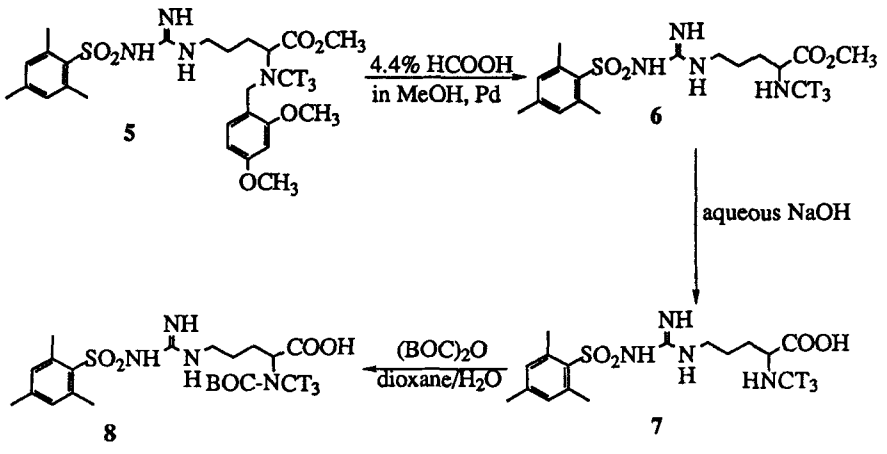
N^2-Substituent on Arginine	Conditions (in DMF, RT, 24 h)	Yield (based on limiting reagent)
BOC	5 eq. MeI, 2 eq. Ag_2O	No Reaction
Benzyl	2.5 eq. MeI, 3 eq. Ag_2O	47%
2,4-Dimethoxybenzyl	2.5 eq. MeI, 2 eq. Ag_2O	67%
2,4-Dimethoxybenzyl	0.29 eq. MeI, 1.05 eq. Ag_2O	87%

In preparation for peptide synthesis, the N-methyl arginine derivative **5** was debenzylated by transfer hydrogenation (Scheme II) followed by saponification and reprotection as the BOC derivative.

Tritiation: Treatment of the dimethoxybenzylarginine derivative **4** with [^{3}H]methyl iodide (1.34 Ci @ 86 Ci/mmol) gave the tritiated N-methyl amino acid (**5**) in 45% radiochemical yield after chromatography (radiochemical purity = 95.1%). Since the [^{3}H]methyl iodide was obtained as a solution in toluene, methylation with silver(I) oxide was carried out in 2:1 DMF/toluene for 41 hours at room temperature. Alkylation with [^{3}H]methyl iodide in this mixed solvent system, rather than in just DMF for 24 hours at room temperature, was not as efficient (87% yield based on methyl iodide in model reactions). A second tritiation using [^{3}H]methyl iodide at slightly lower specific activity (64 Ci/mmol) gave **5** in 59% yield after chromatography (radiochemical purity = 92.1%). Transfer hydrogenation followed by saponification and HPLC purification gave N^G-mesitylenesulfonyl-[^{3}H]methylarginine (**7**) in 71% yield from **5** at a radiochemical purity of 95.5%.

The tritiated N-methyl arginine was protected as its BOC derivative (**8**) in 93% yield by treatment with di-*t*-butyl-dicarbonate in 2:1 dioxane/water. This tritiated BOC arginine was

Scheme II



then incorporated into SK&F 107260-[^{3}H] via standard solution phase peptide synthesis. The final peptide was obtained at a specific activity of 86 Ci/mmol and a radiochemical purity of 95.4% after HPLC purification.

Conclusion

A convenient route for the preparation of N^2-methyl amino acids, labelled with tritium in the methyl group, has been worked out. A tritiated N^2-methylarginine derivative has been synthesized at high specific activity by alkylation of an N^2-2,4-dimethoxybenzylarginine derivative with [^{3}H]methyl iodide according to the method of Olsen (5). Debenzylation, followed by protection as the BOC derivative, gave a tritiated amino acid suitable for incorporation into the cyclic peptide peptide SK&F 107260-[^{3}H]. This tritiated peptide is currently being used in receptor binding assays of various fibrinogen receptor antagonists.

Experimental Section

Methyl N^G-mesitylenesulfonyl arginate (2)

N^G-Mesitylenesulfonyl arginine (**1**, 550 mg, 1.54 mmol) was dissolved in 80 mL of dry methanol (distilled from magnesium methoxide prior to use). Gaseous hydrogen chloride was bubbled through the solution for two minutes. The acidic solution was then heated at reflux for three hours. The solution was concentrated *in vacuo* and purified by flash chromatography (Baker 40 μm silica gel, eluted with 70:30:1 ethyl acetate/methanol/triethylamine). This procedure gave 479 mg (84%) of methyl N^G-mesitylenesulfonyl arginate **2** as a clear oil which was used directly in the next reaction. ^{1}H-NMR($CDCl_3$) δ1.45-1.72 (m, 4H, methylene **H**), 2.26 (s, 3H, CH_3 *para* to SO_2), 2.64 (s, 6H, CH_3 *ortho* to SO_2), 3.12-3.20 (m, 2H, $NHCH_2$), 3.42-3.51 (m, 1H, αCH), 3.70 (s, 3H, $COOCH_3$), 6.88 (s, 2H, ArH).

Methyl N^2-(2,4-dimethoxybenzyl)-N^G-(mesitylenesulfonyl)-arginate (4)

Methyl N^G-mesitylenesulfonyl arginate (**2**, 479 mg, 1.29 mmol) was dissolved in 10 mL of dry methanol (distilled from magnesium methoxide prior to use). To this solution was added 479 mg of activated 3Å molecular sieves. After 10 minutes, 2,4-dimethoxybenzaldehyde (216 mg, 1.29 mmol) was added to the mixture which was then stirred for two hours at room temperature under an argon atmosphere. The mixture was cooled to 0°C and sodium cyanoborohydride (89 mg, 1.42 mmol) was added. The reaction was stirred 18 hours at room temperature, filtered through Celite, and concentrated to an oil *in vacuo*. The crude product was purified by flash chromatography (Baker 40 μm silica gel, eluted with 90:10 ethyl acetate/methanol). The product was obtained as an off-white gummy solid (481 mg, 72%). ^{1}H-NMR($CDCl_3$) δ1.72-2.06 (m, 4H, methylene **H**), 2.43 (s, 3H, CH_3 *para* to SO_2), 2.82 (s, 6H, CH_3 *ortho* to SO_2), 3.20-3.41 (m, 2H, $NHCH_2$), 3.43-3.50 (m, 1H, αCH), 3.87 (s, 3H, $COOCH_3$),4.02 (s, 2H, $ArCH_2NH$), 6.58-6.65 (m, 2H, ArH *ortho* to ArOMe), 7.05 (s, 2H, ArH), 7.25 (d, 1H, J=8.1Hz, ArH *ortho* to CH_2NH). Analysis calculated for $C_{25}H_{36}N_4O_6S.H_2O$: C: 55.74,

H: 7.11, N: 10.40; Found: C: 56.16, H: 6.75, N: 10.32. Mass spectrum (DCI/CH_4) m/z (relative intensity) 521 (69, M+H$^+$), 399 (11), 383 (11), 372 (14), 371 (77), 301 (15), 289 (18), 151 (100).

Methyl N^2-(2,4-dimethoxybenzyl)-N^2-[^{3}H]methyl-N^G-(mesitylenesulfonyl)-arginate (5)

Methyl N^2-(2,4-dimethoxybenzyl)-N^G-(mesitylenesulfonyl)-arginate (**4**, 19 mg, 36.5 mmol) and silver(I) oxide (10 mg, 43.2 μmol, Aldrich) were placed in an oven-dried 3 mL Reacti-Vial. [^{3}H]Methyl iodide (1342 mCi, American Radiolabeled Chemicals), in 1 mL of toluene in a break-seal ampoule, was frozen in liquid nitrogen to condense all the [^{3}H]methyl iodide and then thawed. The procedure was repeated twice. The break seal was broken under an argon atmosphere and the solution was transferred by pipet to the Reacti-Vial. The ampoule was rinsed with dry dimethylformamide and the rinsings were added to the reaction mixture. The vial was completely filled with DMF and placed on a peptide shaker for 41 hours. The solution was then filtered through Celite, the vial rinsed with 2 mL of dry DMF, and the rinsings filtered and added to the filtrate. The solvents were removed by vacuum transfer. The DMF/toluene distillate contained 232 mCi. The residue was dissolved in 1 mL of ethyl acetate and found to contain 1110 mCi. This crude product was purified by column chromatography (Baker 40 μm flash silica gel, eluted with ethyl acetate). The product fractions were combined and found to contain 601 mCi of methyl N^2-(2,4-dimethoxybenzyl)-N^2-[^{3}H]methyl-N^G-(mesitylenesulfonyl)-arginate **5** at a radiochemical purity of 95.1% by HPLC (Beckman Ultrasphere ODS column, 4.6mm ID x 25cm, eluted at 1 mL/min with 65:35:0.1 water/acetonitrile/trifluoroacetic acid, R_t= 17.0 minutes). The ethyl acetate was removed *in vacuo* and the product immediately used in the next reaction. A second tritiation using 1 Ci of [^{3}H]methyl iodide at 64 Ci/mmol (Du Pont NEN) gave **5** in 59% yield (590 mCi) after chromatography (radiochemical purity = 92.1%).

Methyl N^2-[^{3}H]methyl-N^G-(mesitylenesulfonyl)-arginate (6)

The 601 mCi of methyl N^2-(2,4-dimethoxybenzyl)-N^2-[^{3}H]methyl-N^G-(mesitylenesulfonyl)-arginate (**5**) obtained above was dissolved in 1 mL of methanol. To this was added 110 μL of formic acid and 5 mg of Pd black (Engelhard). The mixture was heated at 60°C under an argon atmosphere for 45 min. The reaction was filtered through Celite, the filtrate was concentrated in vacuo, and the residue was dissolved in 5 mL of 95% ethanol. This procedure gave 560 mCi of methyl N^2-[^{3}H]methyl-N^G-(mesitylenesulfonyl)-arginate (**6**) at a radiochemical purity of 89.3% (Beckman Ultrasphere ODS column, 4.6mm ID x 25cm, eluted at 1 mL/min with 73:27:0.1 water/acetonitrile/trifluoroacetic acid, retention time = 15.1 minutes).

N^2-[^{3}H]Methyl-N^G-(mesitylenesulfonyl)-arginine (7)

Methyl N^2-[^{3}H]methyl-N^G-(mesitylenesulfonyl)-arginate **6** obtained in the previous step (560 mCi) was dissolved in 1 mL of 95% ethanol. To this was added 55 μL of 1N NaOH (55 μmol) and the reaction mixture was kept at room temperature for 5 hours. HPLC analysis (Beckman Ultrasphere ODS column, 4.6mm I.D. x 25cm, eluted at 1

mL/min with 73:27:0.1 water/acetonitrile/trifluoroacetic acid) showed 86.9% product (retention time = 7.1 minutes) and 3.7% starting material (retention time = 15.1 min). The reaction mixture was then neutralized with 4.3 µL of trifluoroacetic acid (55 µmol). The reaction mixture was taken to dryness by vacuum transfer and the residue was redissolved in 1 mL of 55:45:0.1 water/acetonitrile/trifluoroacetic acid and purified by semi-preparative HPLC on a LiChrosorb RP-18 column (10mm I.D. x 25cm) eluted with 55:45:0.1 water/acetonitrile/trifluoroacetic acid at 3 mL/min with UV detection at 220 nm. The acetonitrile was removed from the product solution *in vacuo* and the remaining aqueous solution was frozen and lyophilized. The residue was dissolved in 3 mL of 95% ethanol. This procedure gave 399 mCi of N^2-[^{3}H]methyl-N^G-(mesitylenesulfonyl)-arginine **7** with a radiochemical purity of 95.5% by HPLC.

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