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Synthesis of [ω-^{3}H-MeBmt^1]-Cyclosporin A

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Summary

[^{3}H]-Cyclosporin A labeled at the first amino acid ([ω-^{3}H-MeBmt^1]CS) has been prepared by tritium gas hydrogenolysis of [*O*-acetyl-ω-bromo-MeBmt^1]CS followed by deprotection with methanolic sodium methoxide. The requisite bromo intermediate was prepared by bromination of [*O*-acetyl-MeBmt^1]CS with *N*-bromosuccinimide. After synthesis and purification, 2.45 mg of [ω-^{3}H-MeBmt^1]CS with a total activity of 327 MBq were produced with a radiochemical purity >97 %, and specific activity of 160 GBq/mmol (4.3 Ci/mmol).

Key Words: cyclosporin A, tritium gas hydrogenolysis

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Introduction

Cyclosporin A (Scheme 1), the cyclic undecapeptide *cyclo*(-MeBmt[1]-α-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeLeu[10]-MeVal[11]-), MeBmt = (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoic acid is widely used nowadays to prevent transplanted organ rejection and for the treatment of various autoimmune diseases (1). In addition, other cyclosporins derived from cyclosporin A by a formal substitution of some amino acids, like [Nva[2]]CS = cyclosporin G, [D-MeVal[11]]CS = cyclosporin H, or [MeIle[4]]CS, exhibit some interesting activities such as immunomodulating (2), selective formyl peptide receptor antagonist activity (3), and anti HIV activity (4), respectively. Due to the presence of aliphatic amino acids only, cyclosporins offer few opportunities for introducing a label without altering their structure. The simplest way to obtain tritiated cyclosporins is the hydrogenation of the MeBmt[1] double bond. However, the dihydroderivatives exhibit quite different bonding and pharmacological properties (1). The synthesis of [^{3}H]-cyclosporin A selectivelly labeled at the position of the second amino acid ([γ-^{3}H-α-Abu[2]]CS) has been described (1) from [Thr[2]]CS = cyclosporin C by the following sequence of reactions *I*→*IV*, Scheme 1 ([Thr[2]]CS → [Ts-*O*-Thr[2]]CS → [γ-I-α-Abu[2]]CS → [γ-^{3}H-α-Abu[2]]CS). However, this route is limited to threonine-containing cyclosporins (5), i.e. cyclosporins C, S, and W, that are relatively rare. The aim of the present paper is to describe a more general labeling procedure suitable for all MeBmt-containing cyclosporins.

Results and Discussion

[^{3}H]-Cyclosporin A labeled at the first amino acid ([ω-^{3}H-MeBmt[1]]CS, *IX*) has been prepared according to the sequence of reactions *V*→*IX*, Scheme 1. Acetylation of cyclosporins with acetic anhydride is a well established procedure leading almost quantitatively to *O*-protected derivatives (5). The second step, bromination to the ω-bromo-derivative ([*O*-acetyl-ω-bromo-MeBmt[1]]CS, *VII*), was originally developed for the preparation of [ω-hydroxy-MeBmt[1]]CS, the human metabolite of cyclosporin A (6). This reaction was found to be less specific, particulary when using smaller quantities of reaction components. Whereas the side products of bromination can be removed as later

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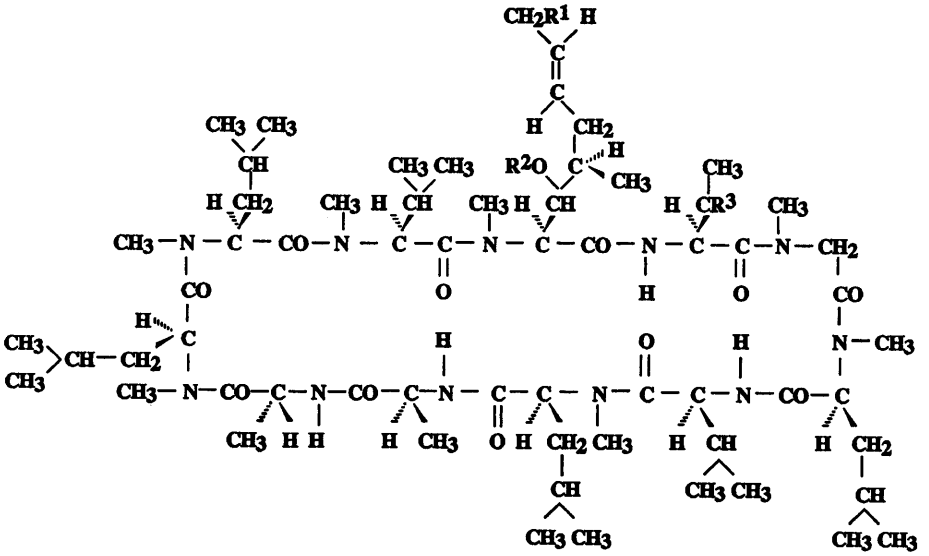
Scheme 1



	R^1	R^2	R^3
I	H	H	OH
II	H	H	CH_3-C_6H_4-O-SO_2-
III	H	H	I
IV	H	H	^{3}H
V	H	H	H
VI	H	CH_3CO	H
VII	Br	CH_3CO	H
VIII	^{3}H	CH_3CO	H
IX	^{3}H	H	H

I ⟶ *II* ⟶ *III* ⟶ *IV* **(ref. 1)**

V $\xrightarrow{(CH_3CO)_2O}$ *VI* $\xrightarrow{(CH_3CO)_2NBr}$ *VII* $\xrightarrow[Et_3N\,/\,CH_3OH]{^3H_2\text{ - }Pd/BaSO_4}$ *VIII* $\xrightarrow{CH_3ONa/CH_3OH}$ *IX* **(this work)**

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as in the final step in the case of the [ω-hydroxy-MeBmt[1]]CS synthesis due to the fact that hydroxy-derivative has quite different chromatographic properties, the purification of [*O*-acetyl-ω-bromo-MeBmt[1]]CS is the key steps in the synthesis of labeled cyclosporin. Since column chromatography on a silica gel did not provide a satisfactory yield and purity of [*O*-acetyl-ω-bromo-MeBmt[1]]CS, two alternative preparative HPLC methods were depeloped (see Experimental). A combination of both methods provided [*O*-acetyl-ω-bromo-MeBmt[1]]CS in a purity >98% (with traces of [*O*-acetyl-MeBmt[1]]CS).

The tritium label was introduced by hydrogenolysis of [*O*-acetyl-ω-bromo-MeBmt[1]]CS, (*VII*, Scheme 1). The exact conditions for hydrogenolysis were estabilished by preliminary experiments with $^2H_2$ in order to achieve nearly quantitative conversion and to avoid hydrogenation of the MeBmt double bond (< 5 %). Methanolic sodium methoxide was used for deacetylation of [ω-^{3}H-*O*-acetyl-MeBmt[1]]CS, (*VIII*, Scheme 1). Final purification of [ω-^{3}H-MeBmt[1]]CS was carried out by HPLC in order to remove traces of [ω-hydroxy-MeBmt[1]]CS, (hydrolysis of residual [*O*-acetyl-ω-bromo-MeBmt[1]]CS) and [dihydro-MeBmt[1]]CS. Since the mixture of $^3H_2/^1H_2$ (15:85, v/v) was used for the reaction, the specific activity of [ω-^{3}H-MeBmt[1]]CS (*IX*) was roughly 160 GBq/mmol (4.3 Ci/mmol). 327 MBq of product were produced with a radiochemical purity >97 %.

Experimental

[O-acetyl-MeBmt[1]]CS (VI)

The solution of cyclosporin A (24.0 g, 20 mmol) and 4-dimethylaminopyridine (3.675 g, 30 mmol) in freshly distilled acetic anhydride (320 ml) was stirred at room temperature for 22 hours. The reaction mixture was then diluted with water (approx. 300 ml) and extracted three times with diethyl ether. The pooled organic extracts were washed sequentially with firstly 0.5 M HCl solution and then water. The organic layer was dried over $MgSO_4$. The solvent was evaporated and the amorphous product was dried under vacuum for 2 days. This crude product (28.2 g) was crystallized from diethyl ether to yield *VI* (10.65 g, 43%, mp 238-9 °C, $[a]_D$ -247° (c = 2.5, MeOH), FAB MS (Finnigan MAT 90, m-nitrobenzylalkohol): m/z 1244.9 $[M+H]^+$, 1184.8 $[MH-CH_3CO_2H]^+$), X-ray crystal structure determination (Enraf-Nonius CAD4): $P2_12_12_1$, a = 12.936(2) Å, b = 15.590(2) Å, c = 36.280(3) Å, the full X-ray data will be published elsewhere (7).

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[O-acetyl-ω-bromo-MeBmt^1]CS (VII)

The acetate *VI* (10 g, 8 mmol) was dissolved in absolute CCl_4 (110 ml), *N*-bromosuccinimide (1.78 g, 10 mmol) and azobisisobutyronitrile (110 mg) were added. The solution was heated in an oil bath under reflux for 2.5 h and kept under anhydrous conditions over nitrogen. The reaction mixture was then allowed to cool, the solvent evaporated and the residue dissolved in diethyl ether. The precipitated solid was filtered off and the filtrate was washed with brine and dried over $MgSO_4$. The yellow waxy product was purified by filtration through a short silica gel column (60 g, diethyl ether/ethyl acetate 4:1) to furnish the crude bromide *VII* (11.6 g). Finally, [*O*-acetyl-ω-bromo-MeBmt^1]CS (*VII*) was obtained by two subsequent preparative HPLC methods (1. Column SGX RPS, 350x25 mm I.D. from Tessek, Czech Republic, isocratic elution with methanol/water 85:15 v/v, temperature 55 °C, flow 7.0 ml/min, det. 240 nm. 2. Column SGX CN 250x25 mm I.D. from Tessek, Czech Republic, isocratic elution with isopropanol/n-hexane, 9:91 v/v, temperature 60 °C, flow 7.0 ml/min, det. 240 nm). Structure verification: FAB MS (Finnigan MAT 90, m-nitrobenzylalkohol): $[M+H]^+$ as a doublet m/z 1322.8/1324.8 ($^{79}Br/^{81}Br$ 1:1). X-ray crystal structure determination (Enraf-Nonius CAD4): $P2_12_12_1$, a = 12.916(3) Å, b = 15.475(4) Å, c = 36.715(7) Å. The full X-ray data and NMR assignment will be published elsewhere (7).

[ω-^{3}H-O-acetyl-MeBmt^1]CS (VIII)

A mixture of [*O*-acetyl-ω-bromo-MeBmt^1]CS (*VII*) (5.5 mg), 10 % $Pd/BaSO_4$ (5.5 mg), triethylamine (40 µl), and methanol (500 µl) was stirred on a gas line for 420 s in a tritium atmosphere ($^3H_2/^1H_2$ 15:85 v/v, 80 kPa). on completion, methanol and triethylamine were distilled off, the residue was suspended in methanol (2 ml) and the catalyst separated by centifugation. The exchangable tritium was removed by evaporation with methanol (2 x 2 ml).

[ω-^{3}H-MeBmt^1]CS (IX)

[ω-^{3}H-*O*-acetyl-MeBmt^1]CS (*VIII*) was dissolved in methanolic sodium methoxide (0.3 ml, 0.25 M) and the mixture allowed to stand for 90 hrs. The reaction was terminated by the addition of acetic acid (12 µl). Final purification was carried out by HPLC (RP column SGX-RPS 250x4 mm, I.D., 7 µm (Tessek, Czech Republic), isocratic elution with acetonitrile/water (66:34 v/v) at 70 °C, flow 1 ml/min, uv detection at 214 nm). The reaction produced 2.45 mg of *IX* at a total radioactivity of 327 MBq and a radiochemical purity >97 %, the specific activity being 160 GBq/mmol (4.3 Ci/mmol).

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