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Antibody Against a Novel, Myriocin (ISP-I)-Based Immunosuppressant, FTY720

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Abstract—An antibody was prepared by immunizing rabbits with an ovalbumin conjugate of 2-amino-2-(2-(4-(4-mercaptobutyl)-phenyl)ethyl)propane-1,3-diol HCl (AMPD-4), which contains the essential structure of the novel immunosuppressant FTY720. As the antibody reacted to not only AMPD-4, but also FTY720, it should be useful for immunoassay of FTY720 in body fluids, tissues and cells. © 2000 Elsevier Science Ltd. All rights reserved.

FTY720 (**1**: 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol HCl),[1] a novel immunosuppressant, was discovered by chemical modification based on the structure–activity relationships[2–4] of ISP-I (**2**),[5–8] a metabolite of the fungus *Isaria sinclairii*. Compound **1** has more potent activity than established drugs such as cyclosporin A and FK506.[9–11] Furthermore, combination therapy with the other drugs showed a remarkable synergistic effect in prolonging skin, cardiac, and renal allograft survival.[9,10,12–14] This is considered to be because **1** has an entirely different action mechanism from the other drugs.[9] In spite of the established therapeutic effectiveness of **1**, little clinical and experimental knowledge is available about its pharmacokinetic properties, disposition, and so on, due to the lack of an antibody against **1**.

The aims of this study were (1) to synthesize 2-amino-2-(2-(4-(4-mercaptobutyl)phenyl)ethyl)propane-1,3-diol HCl, AMPD-4 (**3**), which contains the essential structure of **1**[4] and a thiol group that can be used to conjugate the molecule to a maleimide-modified carrier protein, (2) to prepare AMPD-4-carrier protein conjugate, and (3) to examine the availability of the AMPD-4-carrier protein conjugate as an antigen to prepare an antibody against **1**.

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Synthesis of 2-amino-2-(2-(4-(4-mercaptobutyl)phenyl) ethyl)propane-1,3-diol HCl (AMPD-4)

AMPD-4 (**3**) was synthesized according to Scheme 1.[15] An ester **4**,[16] which was prepared from 4-phenylbutyric acid, was bromoacetylated to give **5**. Compound **5** was reduced with triethylsilane in trifluoroacetic acid to afford **6**,[17] which was converted to **7**. The iodide **7** was condensed with diethyl acetamidomalonate to give the triethylester **8**. Reduction of triester **8** with sodium borohydride, followed by treatment with 2,2-dimethoxypropane, afforded a protected monoester **9** as a major product and an alcohol **10** as a minor product. The ester **9** was reduced with lithium aluminum hydride to give the alcohol **10**, which was converted to a tosylate (**11**). Compound **11** was treated with potassium thioacetate to give the thioacetate **12**, and then acid hydrolysis yielded AMPD-4 (**3**).

Preparation of AMPD-4-protein conjugates (Scheme 2)

Maleimide groups were introduced into ovalbumin (OVA) and bovine serum albumin (BSA) by using ε-maleimidocaproic acid *N*-hydroxysuccimide ester (EMCS),[18] and the average numbers[19] per protein molecule were 8.4 and 10.1, respectively. AMPD-4-protein conjugates were prepared by Michael-type addition of the thiol group of **3** with the maleimide moieties. The average numbers of molecules of **3** introduced per protein molecule were 6.8 and 9.6, respectively, which were calculated from the

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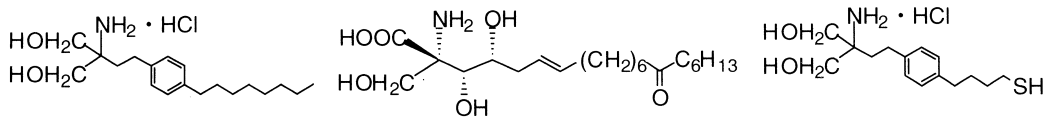
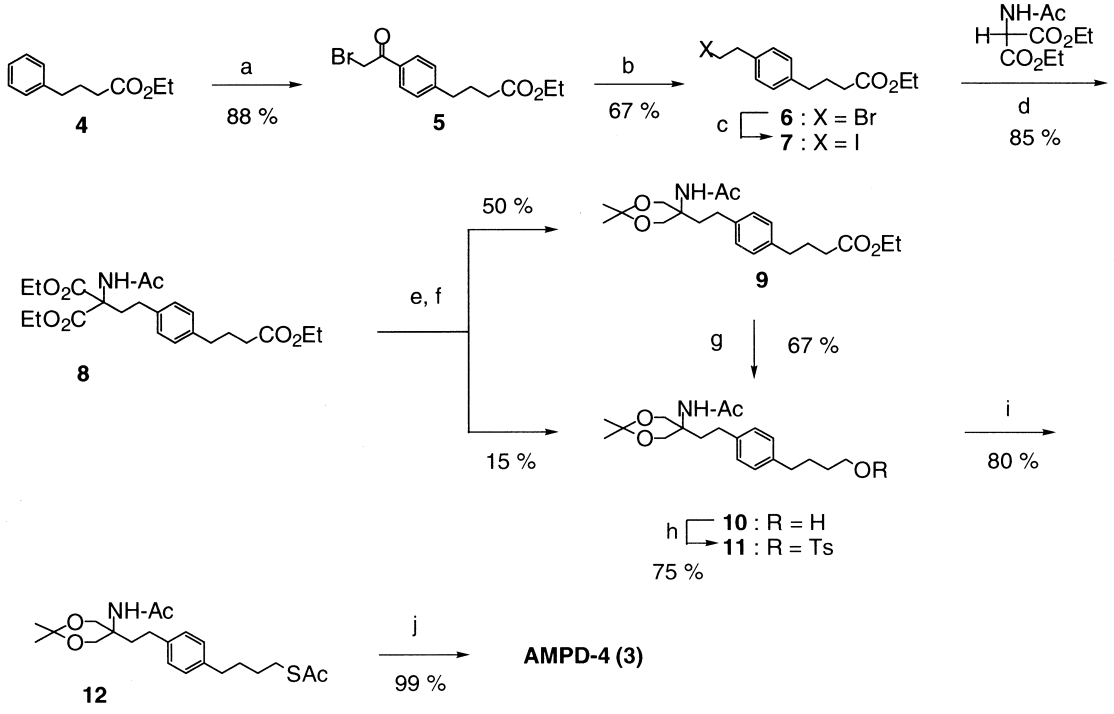


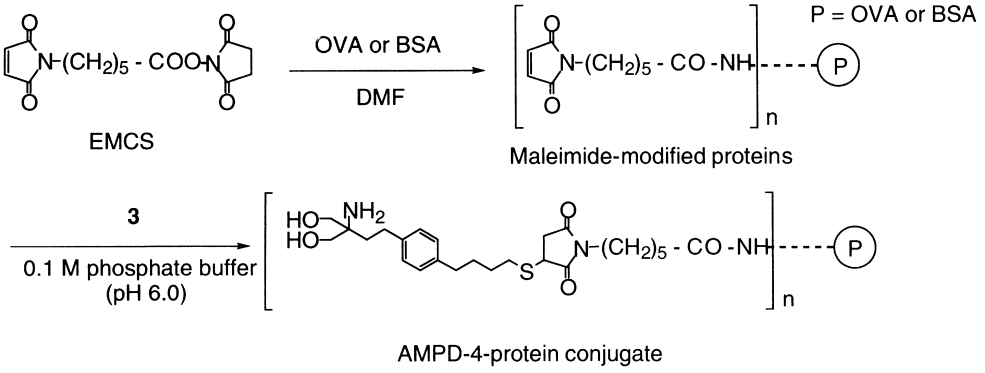
decrease in the number of maleimide groups.[19] The residual maleimide moieties were protected with 2-mercaptoethylamine and purified by gel filtration on a column of Sephadex G-25 to give AMPD-4-protein conjugates.

Immunization of rabbits with AMPD-4-OVA conjugate

Three rabbits (Japanese White, 2.7–2.8 kg) were intracutaneously injected with AMPD-4-OVA conjugate (0.1 mg) in Freund's complete adjuvant three times at three-week intervals. As shown in Figure 1, anti-AMPD-4 IgG antibody could be detected in sera from every rabbit by enzyme-linked immunosorbent assay (ELISA), in which an AMPD-4-BSA-coated solid phase was incubated with test sera and, after washing, with horseradish peroxidase (HRP)-labelled goat (anti-rabbit IgG) Fab'. This result showed that AMPD-4-OVA conjugate was effective as an antigen.



Scheme 1. (a) $BrCH_2COCl$, $AlCl_3$, $CHCl_3$; (b) Et_3SiH, TFA; (c) NaI. 2-butanone; (d) NaH, DMF-THF; (e) $NaBH_4$, MeOH; (f) $(CH_3)_2C(OCH_3)_2$, TsOH; (g) $LiAlH_4$, Et_2O; (h) TsCl, Et_3N, CH_2Cl_2; (i) $KSCOCH_3$, EtOH; (j) 10% HCl (aq), EtOH.



Scheme 2.

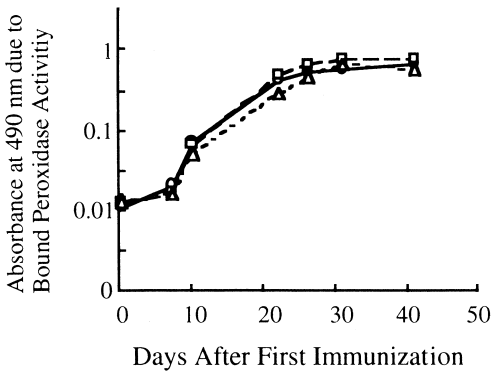


Figure 1. Three rabbits (○, △, □) were inoculated with AMPD-4-OVA. Anti-AMPD-4 IgG was measured by ELISA using AMPD-4-BSA as an antigen.

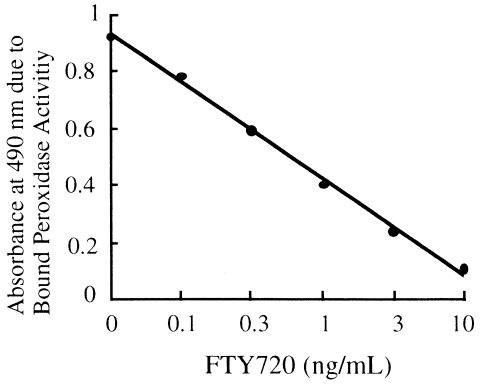


Figure 2. Standard curve for FTY720 (**1**) in competitive enzyme immunoassay using HRP-labelled anti-AMPD-4-OVA Fab'.

Specificity of the antibody

An enzyme immunoassay for **1** based on a competitive format was conducted to examine the applicability of the antibody for the assay of **1**. In brief, HRP-labelled anti-AMPD-4-OVA Fab' was incubated with **1** and trapped on an AMPD-4-BSA-coated solid phase. The solid phase was washed, and the bound HRP activity was detected. A standard curve for **1** obtained by this method is shown in Figure 2. This result clearly indicates that the antibody prepared, reacts not only with **3**, but also with **1**. Therefore, it should be applicable to study the pharmacokinetic properties and disposition of **1**. The enzyme immunoassay method for **1** containing the specificity will be reported in detail elsewhere.

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15. All new compounds in this communication gave analytical and spectroscopic data in full accord with their assigned structures. Compound **3**: colorless plates. Mp 105–113 °C (dec). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550–3150, 3150–2400. ^1H NMR (DMSO-d_6) δ 1.53 (2H, m), 1.62 (2H, m), 1.76 (2H, m), 2.23 (1H, t, J = 8.0 Hz), 2.46–2.55 (4H, m), 2.59 (2H, m), 3.52 (each 2H, d, J = 5.0 Hz), 5.36 (2H, t, J = 5.0 Hz), 7.11 (4H, br s), 7.82 (3H, br s). FAB-MS (negative) m/z: 318, 320 $(M-1)^+$. Anal. calcd for $C_{15}H_{26}NO_2SCl$: C, 56.32; H, 8.19; N, 4.38. Found: C, 56.37; H, 8.09; N, 4.49. Compound **10**: colorless needles. Mp 106–108 °C. IR ν_{max} (KBr) cm^{-1}: 3440, 1680. ^1H NMR ($CDCl_3$) δ: 1.42 (6H, s), 1.55 (2H, m), 1.65 (2H, m), 2.01 (3H, s), 2.05 (2H, m), 2.51 (2H, m), 2.63 (2H, t, J = 7.4 Hz), 3.66, 3.96 (each 2H, d, J = 12.0 Hz), 5.69 (1H, br s), 7.07 (4H, m). EI-MS m/z: 349 (M^+). Anal. calcd for $C_{20}H_{31}NO_4$: C, 68.74; H, 8.94; N, 4.01. Found: C, 68.66; H, 8.66; N, 3.83.
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