Synthesis of a Dibenz[*b,e*]oxepin–Bovine Serum Albumin Conjugate for Radioimmunoassay of KW-4679 ((*Z*)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic Acid Hydrochloride)

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(*Z*)-11-[3-(Dimethylamino)propylidene]-2-(methoxycarbonyl)methyl-6,11-dihydrodibenz[*b,e*]oxepin-9-acrylic acid (**5**) was prepared for application to the radioimmunoassay of KW-4679 (**1**, (*Z*)-11-[3-(dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic acid hydrochloride). The acrylic acid moiety in the 9-position of **5** was employed for coupling with an amino group of bovine serum albumin (BSA) to provide **17**. Subsequently, the conjugate **17** was treated with aqueous NaOH to hydrolyze the terminal methoxycarbonyl group in the 2-position of the BSA conjugated **5**. Antiserum raised against the antigenic BSA-conjugate **4** finally obtained was specific for **1**.

Keywords radioimmunoassay; KW-4679; bovine serum albumin; conjugate; hapten; 6,11-dihydrodibenz[*b,e*]oxepin; palladium-catalyzed coupling

We recently reported (*Z*)-11-[3-(dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic acid hydrochloride (**1**, KW-4679) as an effective and orally active antiallergic agent,¹⁾ which is now under clinical evaluation (Fig. 1).

Based on its potency in experimental animal models, the dosage of **1** in clinical studies was estimated to be 1–20 mg/man. Therefore, radioimmunoassay was considered to be the most suitable method to determine the level of **1** in human plasma. This assay required the preparation of an effective antibody, thus the antigenic complex **3** was synthesized. Antiserum raised against the antigen **3** showed a high affinity for **1**. However, this antiserum was not specific enough for **1**; a considerable cross reactivity for **2** (35.7%), a metabolite detected in rats administered with **1**, was observed.²⁾ Therefore, we attempted to synthesize a new bovine serum albumin (BSA)

conjugate **4**, in which the hapten is coupled to BSA in the 9-position of **1** to obtain the specificity. In this paper we report the synthesis of **5** and its subsequent conversion into **4**.

In the first instance, a 9-bromo derivative, **6**, was selected as the substrate for palladium-catalyzed C–C bond formation (Fig 2). Although carbonylation³⁾ of **6** in methanol provided **7** in a poor yield (<10%), treatment of **6** with ethyl acrylate in the presence of Pd(OAc)₂ and 1,3-bis(diphenylphosphino)propane (dppp) afforded **8** via olefin insertion⁴⁾ in a moderate yield (33%).⁵⁾ A protective group of the acrylic acid must be cleaved selectively for the conjugation with BSA. Thus the ethyl ester in **8** was replaced with (2-trimethylsilyl)ethyl ester (**9**). Compound **9** was prepared from **6** and (2-trimethylsilyl)ethyl acrylate⁶⁾ by the same method as described above (66%) and was led to the half ester **10** almost quantitatively (*n*-Bu₄NF in tetrahydrofuran (THF)).

Based on the results of the model experiments described above, a multi-functionallized 6,11-dihydrodibenz[*b,e*]oxepin derivative (**5**) was synthesized (Chart 1). Although compound **12** was obtained by the Wittig reaction of **11** and [3-(dimethylamino)propyl]triphenylphosphonium bromide hydrobromide as described in our previous report,¹⁾ palladium-catalyzed coupling with (2-trimethylsilyl) ethyl acrylate provided a complex mixture. Moreover, we failed in the Wittig olefination of **9** to provide **16**. Therefore, compound **11** was converted into **13** by the treatment with the ylide generated from [3-[(tetrahydro-2*H*-pyran-2-yl)-oxy]propyl]triphenylphosphonium bromide,⁷⁾ and by subsequent simultaneous cleavage of the tetrahydropyranyl ether and esterification of the carboxyl group (*p*-toluenesulfonic acid/MeOH, reflux). The crude **13** obtained was a mixture of geometrical isomers (*E/Z* = 1/2) and was

Fig. 1

Fig. 2

Chart 1

purified by recrystallization from diisopropyl ether to provide the *Z*-isomer (**13**). The geometry around the 11-position was determined by nuclear Overhauser effect (NOE) experiments on proton nuclear magnetic resonance ($^1\text{H-NMR}$).⁸⁾ The bromide **13** underwent the palladium-catalyzed olefin insertion to afford **14** (15%). Compound **14** was treated with methanesulfonyl chloride to provide **15**, which was converted to **16** with dimethylamine (overall, 56%). Treatment of **16** with *n*-Bu₄NF afforded **5** in a yield of 50%. Detectable isomerization of the double bond in the 11-position was observed during the conversion from **13** to **5**.⁹⁾

Coupling of **5** with BSA was accomplished by means of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI). The resulting conjugate **17** was treated with aqueous NaOH (pH 12) at 45°C for 1 h in order to obtain **4**. The reaction conditions were established on the basis of the observation that the methyl ester in the 2-position of **5** was completely hydrolyzed in an aqueous solution (pH 10, room temperature, <30 min). Although we did not confirm the structure of the hapten in the finally obtained antigen **4**, the antiserum raised against **4** showed high affinity for **1**. Additionally, this antiserum exhibited negligible cross reactivities for **2** and the methyl ester of **1** (1.1% and 3.8%, respectively).

In conclusion, a multi-functionallized 6,11-dihydrodibenz[*b,e*]oxepin derivative (**5**) was synthesized *via* palladium-catalyzed olefin insertion. Compound **5** was conjugated with BSA from the 9-position of the dibenzoxepin ring system. Subsequently, the resulting BSA-conjugate **17** was treated with aqueous NaOH to afford the final product **4**. The application of this newly prepared antigen **4** to the radioimmunoassay of KW-4679 has been successful and the results will be published in a separate paper.

Experimental

General Procedures Melting points were determined with a Büchi-510 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-400 spectrometer. $^1\text{H-NMR}$ spectra were recorded on a JEOL PMX-60 (60 MHz), a Hitachi R-90H (90 MHz), or a JEOL GX-270 (270 MHz) spectrometer with Me₄Si as the internal standard. Mass spectra (MS) were recorded on a JEOL D300 mass

spectrometer. Elemental analyses were performed by the analytical department of our laboratories. For column chromatography, silica gel: Kieselgel 60 (Merck, 70–230 or 230–400 mesh) and highly porous synthetic resin: Diaion HP-40 (Mitsubishi Chem. Ind. Co., Ltd.) were used. *E/Z* ratios were measured by high performance liquid chromatography (HPLC), column: YMC A-312 (ODS, 6 mm × 150 mm), eluent: 0.01 M octanesulfonic acid in MeOH/H₂O (2/1).

9-Bromo-11-oxo-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic Acid Methyl Ester (6) and 9-Bromo-11-oxo-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic Acid (11) Compound **6** was prepared from methyl 4-hydroxyphenylacetate and 6-bromophtalide by a similar method as described in our previous report.¹⁰⁾ Alkaline saponification of **6** afforded **11**. **6**: oil. $^1\text{H-NMR}$ (CDCl₃) δ : 3.64 (s, 2H), 3.70 (s, 3H), 5.11 (s, 2H), 7.01 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 1H), 7.3–7.7 (m, 3H), 8.04 (dd, *J* = 2.2, 5.9 Hz, 1H). MS *m/z*: 360 (*M*⁺), 362 (*M*⁺ + 2). Anal. Calcd for C₁₇H₁₃BrO₄: C, 56.53; H, 3.63. Found: C, 56.44; H, 3.73. **11**: mp 192–194°C. $^1\text{H-NMR}$ (CDCl₃) δ : 3.62 (s, 2H), 5.27 (s, 2H), 7.06 (d, *J* = 8.3 Hz, 1H), 7.4–7.6 (m, 2H), 7.7–8.0 (m, 3H). MS *m/z*: 346 (*M*⁺), 348 (*M*⁺ + 2). Anal. Calcd for C₁₆H₁₁BrO₄: C, 55.36; H, 3.19. Found: C, 55.26; H, 3.25.

(*Z*)-11-[3-(Hydroxy)propylidene]-9-bromo-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic Acid Methyl Ester (13) [3-[(Tetrahydro-2*H*-pyran-2-yl)oxy]propyl]triphenylphosphonium bromide⁷⁾ (5.0 g, 10.3 mmol) was suspended in THF (30 ml). To the suspension *n*-BuLi solution in hexane (1.55 N, 6.4 ml, 9.9 mmol) was added at 0°C under Ar atmosphere, and the mixture was stirred under the same conditions for 30 min. A THF solution (25 ml) containing **11** (1.3 g, 3.73 mmol) was added dropwise. The resultant mixture was stirred at room temperature overnight. To the reaction mixture, 1 N HCl and AcOEt were added. The organic phase was separated, washed with brine, dried, and evaporated. The residue was diluted with MeOH (100 ml) containing a catalytic amount of *p*-TsOH and the solution was stirred at room temperature for 2.5 h. The medium was neutralized with sat. NaHCO₃ and then concentrated. The residue was diluted with AcOEt and the solution was washed with brine, dried, and evaporated. The crude product was chromatographed on silica gel (hexane–AcOEt, 2:1) and subsequently recrystallized from diisopropyl ether to give 0.88 g (59%) of **13** as a solid, mp 145–146°C. $^1\text{H-NMR}$ (CDCl₃) δ : 2.5–3.8 (m, 2H), 3.53 (s, 2H), 3.69 (s, 3H), 3.81 (t, *J* = 6.5 Hz, 2H), 5.13 (brs, 2H), 5.77 (t, *J* = 7.6 Hz, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 6.9–7.5 (m, 5H). MS *m/z*: 402 (*M*⁺), 404 (*M*⁺ + 2). Anal. Calcd for C₂₀H₁₉BrO₄: C, 59.57; H, 4.75. Found: C, 59.48; H, 4.89.

(*Z*)-11-[3-(Hydroxy)propylidene]-2-(methoxycarbonyl)methyl-6,11-dihydrodibenz[*b,e*]oxepin-9-acrylic Acid (2-Trimethylsilyl)ethyl Ester (14) A mixture of **13** (0.33 g, 0.82 mmol), (2-trimethylsilyl)ethyl acrylate⁶⁾ (0.53 g, 3.1 mmol), tributylamine (0.32 ml, 1.3 mmol), Pd (OAc)₂ (0.01 g, 0.041 mmol), and 1,3-bis(diphenylphosphino)propane (0.03 g, 0.073 mmol) was heated at 130°C for 16 h. After being cooled, the reaction mixture was diluted with Et₂O and AcOEt. The organic solution was washed successively with 1 N HCl, sat. NaHCO₃, and brine, dried and concentrated. The residue was chromatographed on silica gel (hexane–AcOEt, 3:1) to give 0.059 g (15%) of **14** as an oil. $^1\text{H-NMR}$ (CDCl₃) δ : 0.07 (s, 9H), 1.16

(t, $J=8.4$ Hz, 2H), 2.4–2.7 (m, 2H), 3.54 (s, 2H), 3.68 (s, 3H), 3.81 (t, $J=6.5$ Hz, 2H), 4.30 (t, $J=8.4$ Hz, 2H), 5.18 (br s, 2H), 5.79 (t, $J=7.5$ Hz, 1H), 6.35 (d, $J=16.0$ Hz, 1H), 6.73 (d, $J=8.4$ Hz, 1H), 6.8–7.4 (m, 5H), 7.57 (d, $J=16.0$ Hz, 1H). High resolution MS m/z : Calcd for $C_{28}H_{34}O_6Si$ 494.2125. Found: 494.2133 (M^+).

Compounds **8** (oil) and **9** (oil) were prepared by a similar method as described above. **8**: 1H -NMR ($CDCl_3$) δ : 1.34 (t, $J=7.1$ Hz, 3H), 3.65 (s, 2H), 3.71 (s, 3H), 4.27 (q, $J=7.1$ Hz, 2H), 5.18 (s, 2H), 6.50 (d, $J=16.3$ Hz, 1H), 7.03 (d, $J=8.4$ Hz, 1H), 7.2–7.7 (m, 3H), 7.71 (d, $J=16.3$ Hz, 1H), 8.0–8.2 (m, 2H). High resolution MS m/z : Calcd for $C_{22}H_{26}O_6$ 380.1260. Found 380.1286 (M^+). **9**: 1H -NMR ($CDCl_3$) δ : 0.07 (s, 9H), 1.08 (t, $J=8.3$ Hz, 2H), 3.57 (s, 2H), 3.63 (s, 3H), 4.30 (t, $J=8.3$ Hz, 2H), 5.17 (s, 2H), 6.41 (d, $J=16.3$ Hz, 1H), 6.94 (d, $J=8.4$ Hz, 1H), 7.1–7.6 (m, 3H), 7.69 (d, $J=16.3$ Hz, 1H), 7.85–8.0 (m, 2H). High resolution MS m/z : Calcd for $C_{25}H_{28}O_6Si$ 452.1655. Found: 452.1678 (M^+).

(Z)-11-[3-(Dimethylamino)propylidene]-2-(methoxycarbonyl)methyl-6,11-dihydrodibenz[*b,e*]oxepin-9-acrylic Acid (2-Trimethylsilyl)ethyl Ester (16) Compound **14** (59 mg, 0.12 mmol) was dissolved in pyridine (2 ml). Methanesulfonyl chloride (0.02 mg, 0.24 mmol) was added at 0°C and the mixture was stirred under the same conditions for 30 min. The reaction mixture was diluted with AcOEt. The organic solution was washed successively with 1 N HCl, sat. $NaHCO_3$, and brine, dried, and evaporated. The crude **15** obtained was used in the next reaction without further purification. A mixture of the crude **15**, 50% Me_2NH in H_2O (0.11 ml, 1.2 mmol) and isopropanol (10 ml) was refluxed for 2.5 h and then concentrated. The residue was diluted with AcOEt. The organic solution was washed with brine, dried, and evaporated. The crude product was chromatographed on silica gel (hexane–AcOEt–triethylamine, 10:10:1) to give 35 mg (56%) of **16** as an oil. 1H -NMR ($CDCl_3$) δ : 0.07 (s, 9H), 1.16 (t, $J=8.1$ Hz, 2H), 2.22 (s, 6H), 2.3–2.8 (m, 4H), 3.52 (s, 2H), 3.66 (s, 3H), 4.30 (t, $J=8.1$ Hz, 2H), 4.9–5.3 (br, 2H), 5.75 (t, $J=7.5$ Hz, 1H), 6.42 (d, $J=16.1$ Hz, 1H), 6.7–7.5 (m, 9H), 7.65 (d, $J=16.1$ Hz, 1H). High resolution MS m/z : Calcd for $C_{30}H_{39}NO_5Si$ 521.2598. Found: 521.2574 (M^+).

(Z)-11-[3-(Dimethylamino)propylidene]-2-(methoxycarbonyl)methyl-6,11-dihydrodibenz[*b,e*]oxepin-9-acrylic Acid (5) Compound **16** (33 mg, 0.063 mmol) was treated with a mixture of $n-Bu_4NF$ solution in THF (1 M, 0.2 ml, 0.2 mmol) and THF (5 ml) at room temperature for 1 h. After being concentrated, the reaction mixture was chromatographed on HP-40 (MeOH– H_2O , 1:1 and then MeOH) to give 13.2 mg (50%) of **5** as an oil. 1H -NMR ($CDCl_3$) δ : 2.37 (s, 6H), 2.5–2.9 (m, 4H), 3.52 (s, 2H), 3.66 (s, 3H), 4.9–5.4 (br, 2H), 5.76 (t, $J=7.5$ Hz, 1H), 6.17 (d, $J=16.1$ Hz, 1H), 6.79 (d, $J=9.0$ Hz, 1H), 7.0–7.4 (m, 5H), 8.5–9.0 (m, 2H). MS m/z : 421 (M^+). Anal. Calcd for $C_{25}H_{27}NO_5$: C, 71.24; H, 6.46; N, 3.32. Found: C, 70.98; H, 6.70; N, 3.12.

Compound **10** (oil) was prepared by a method similar to that described above. 1H -NMR ($CDCl_3$) δ : 3.65 (s, 2H), 3.70 (s, 3H), 5.19 (s, 2H), 6.50

(d, $J=16.0$ Hz, 1H), 7.03 (d, $J=8.4$ Hz, 1H), 7.2–8.1 (m, 5H), 7.67 (d, $J=16.0$ Hz, 1H). Anal. Calcd for $C_{20}H_{16}O_6$: C, 68.18; H, 4.58. Found: C, 67.89; H, 4.76.

Preparation of Antigen 4 To a mixture of **5** (5 mg, 0.012 mmol) in H_2O (0.2 ml), a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3 mg, 0.016 mmol) in H_2O (0.03 ml) and a solution of bovine serum albumin (5 mg) in H_2O (0.5 ml) were added. The resultant mixture was stirred at room temperature for 1 h. The crude conjugate **17** was dialyzed against saline (5 l) overnight and centrifuged at 2700 rpm for 10 min. The resultant supernatant was adjusted to pH 12 with aqueous NaOH and warmed at 45°C for 1 h. After being neutralized with 1 N HCl, the crude conjugate **4** was dialyzed twice against saline (5 l) for 24 h.

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References and Notes

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