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 radiommunoassay 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**.

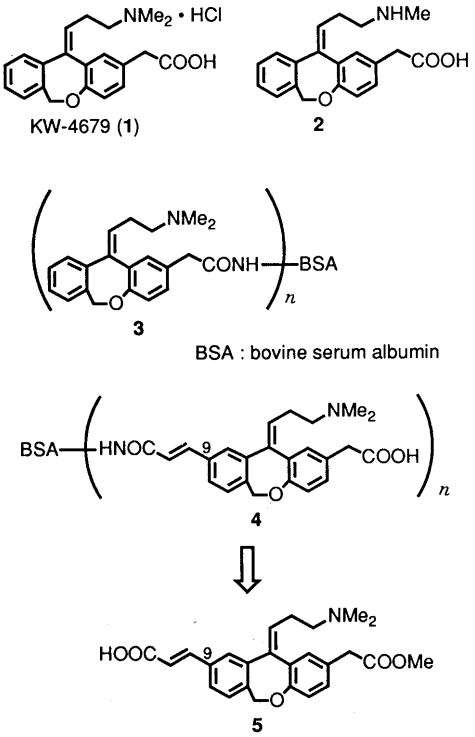
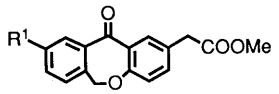


Fig. 1

In the first instance, a 9-bromo derivative, **6**, was selected as the substrate for palladium-catalyzed C–C bond formation (Fig 2). Although carbonylation[3] 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 insertion[4] 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 acrylate[6] by the same method as described above (66%) and was led to the half ester **10** almost quantitatively (n-Bu_4NF 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 acylate 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



6: R^1 = Br
7: R^1 = COOMe
8: R^1 = CH=CHCOOEt
9: R^1 = CH=CHCOOCH$_2$CH$_2$SiMe$_3$
10: R^1 = CH=CHCOOH

Fig. 2

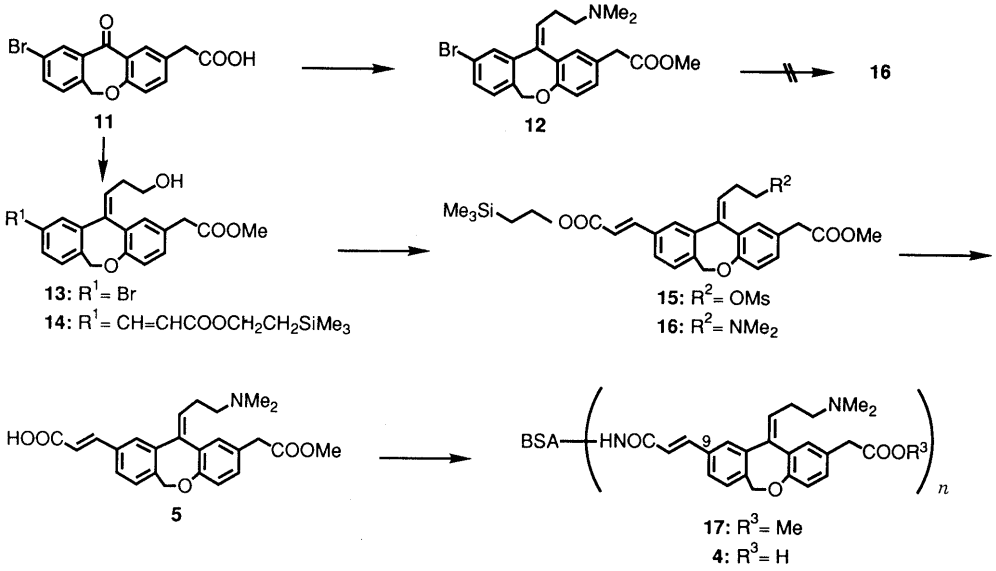


Chart 1

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}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*-Bu_4NF 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-ethycarbodiimide 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}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 Me_4Si 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/H_2O (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 descrived in our previous report.[10] Alkaline saponification of **6** afforded **11**. **6**: oil. ^{1}H-NMR ($CDCl_3$) 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 $C_{17}H_{13}BrO_4$: C, 56.53; H, 3.63. Found: C, 56.44; H, 3.73. **11**: mp 192—194 °C. ^{1}H-NMR ($CDCl_3$) 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 $C_{16}H_{11}BrO_4$: 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 bromide[7] (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. $NaHCO_3$ 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}H-NMR ($CDCl_3$) 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 (br s, 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 $C_{20}H_{19}BrO_4$: 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 acrylate[6] (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 Et_2O and AcOEt. The organic solution was washed successively with 1 N HCl, sat. $NaHCO_3$, 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}H-NMR ($CDCl_3$) 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**: ^{1}H-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 $C_{22}H_{20}O_6$ 380.1260. Found 380.1286 (M^+). **9**: ^{1}H-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–triethyamine, 10 : 10 : 1) to give 35 mg (56%) of **16** as an oil. ^{1}H-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. ^{1}H-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. ^{1}H-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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