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# Facile Synthesis of 3-Methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one: Application to the Acid-Labile Peptide Amide Linker Containing the 10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene Moiety

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**The facile synthesis of 3-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (5) *via m*-methoxystilbene-2-carboxylic acid (3) was developed for the preparation of the mild acid labile peptide amide linker containing 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl group for Fmoc (9-Fluorenylmethoxycarbonyl)-solid phase peptide synthesis.**

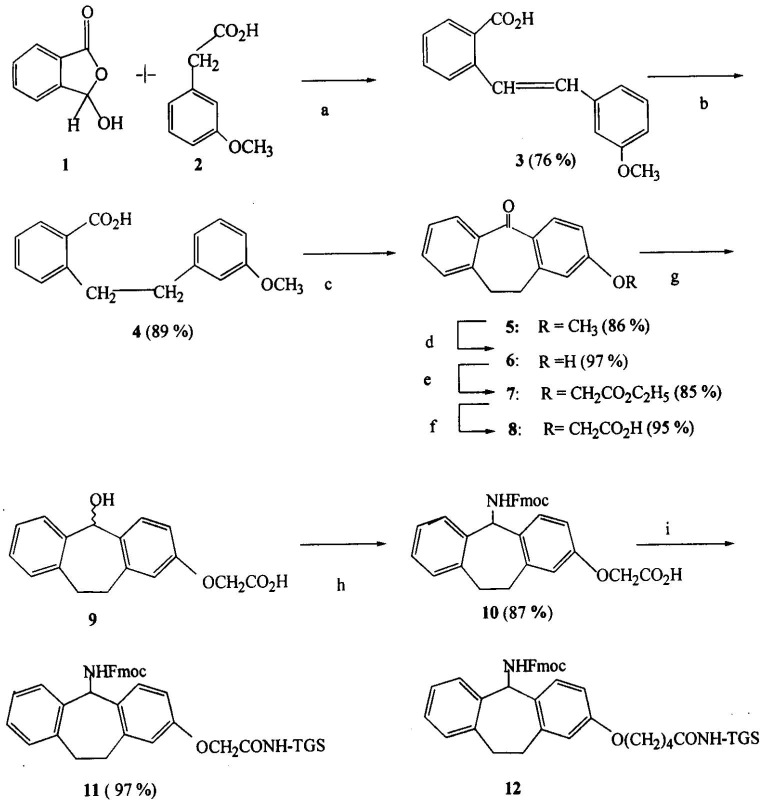
**Key words** peptide amide; linker; 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl group; Fmoc (9-Fluorenylmethoxcarbonyl)-solid phase peptide synthesis

Since C-terminal peptide amides are incorporated into many biologically active hormones and neuro transmitters, synthesis by solid phase peptide synthesis (SPPS) has attracted much attention. One of the most crucial steps in SPPS is cleavage of the peptide from the solid support. SPPS requires that the linkage between the peptide being assembled and the support remains intact throughout many reaction cycles and then is selectively cleaved in the final step. It was previously noted that the design of novel linker for Fmoc (9-Fluorenylmethoxycarbonyl)-SPPS would require the rupture of the amino carbon–nitrogen linkage by mild acid treatment.[1] 10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl group was used for selective protection when a side-chain carboxyl group as well as –OH, –NH, and –SH groups were present.[2] It can be cleaved by hydrogenolysis and by dilute aqueous acid treatment at ambient temperature. The reaction may proceed through a resonance-stabilized carbonium ion or a kinetically equivalent mechanism.[3] Carbonium ions of the type derived from an alcohol, amine, and thiol would be resonance-stabilized from two phenyl rings thus giving a total of seven resonance-stabilized structures with the positive charge distributed over the tricyclic nucleus. Seven-membered rings are well known for their relative ease of carbonium ion formation.[4] The quantitative range of relative stabilities in the di- and triphenylmethyl series, as shown by measurement of the $pK_{R+}$ values, would have predicted the order of stability to be 5*H*-dibenzo-[*a*,*d*]cyclohepten-5-yl > trityl > 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl > benzhydryl group.[2,5] In a previous paper, novel linkers for peptide amide formation under mild conditions during Fmoc-SPPS were designed and prepared based on the 10.11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl group and 5*H*-dibenzo[*a*,*d*]-cyclohepten-5-yl group,[6] as a resulted relative stabilities of the incipient carbonium ion formed upon acid treatment as predicted by $pK_{R+}$ values.

However, the previous synthetic process for these linkers included the preparation of 2-(*m*-methoxyphenethyl)-benzoic acid (4) by catalytic hydrogenolysis of 3-(*m*-methoxybenzylidene)phthalide using Raney Ni (4 kg/cm², 50 °C), which was not suitable for a large scale preparation of 2-methoxy-10,11-dihydro 5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**5**). The present paper describes a new, facile synthesis of **5** which is readily converted to the 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-derived linker, and should also be amenable to preparation of the mild acid labile protecting group for the guanidino function of Fmoc-arginine.[7]

Synthesis of *m*-methoxystilbene-2-carboxylic acid (3), the precursor of the key intermediate, 2-methoxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**5**), was reported using the Wittig reaction of the corresponding phosphorane derived from 2-methylbenzoate with *m*-anisaldehyde.[8] The new, more simple one-step synthesis of this intermediate is more readily amenable to scale-up. The fusion reaction of 2-carboxybenzaldehyde (**1**) and *m*-methoxyphenylacetic acid in the presence of sodium acetate gave rise to *m*-methoxystilbene-2-carboxylic acid (**3**) with concomitant evolution of carbon dioxide in 76% yield.[9] Catalytic hydrogenation of **3** with 5% Pd–C gave 2-(*m*-methoxyphenethyl)benzoic acid (**4**), which was then cyclized by treatment of polyphosphoric acid (PPA) at 145—150 °C to give **5** in 86% yield as a pure regioisomer. This tricyclic compound was converted to the demethylation product (**6**) by treatment with $AlCl_3$ in 97% yield, as described in a previous paper.[6] In order to introduce this tricyclic structure moiety onto polymer supports, the resulting OH group was alkylated with ethyl bromoacetate rather than ethyl 5-bromovalerate,[6] after considering scale-up issue in the presence of *tert*-BuOK in *N*,*N*-dimethylformamide (DMF) solution, followed by hydrolysis with aqueous NaOH to give the corresponding acid **8**. Reduction of **8** with $NaBH_4$ gave the unstable alcohol (**9**), which easily formed the carbonium cation in the presence of a catalytic amount of *p*-toluenesulfonic acid (PTS) in DMF solution, that was then trapped with 9-fluorenylmethoxycarbamate (Fmoc-$NH_2$) *in situ* to give the 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclopentene linker derivative (**10**) in 40% overall yield.

The desired linker-resin coupling was achieved by quantitatively introducing **10** onto polyethylene glycol–polystyrene (PEG–PS) graft copolymer functionalized with an amino group[10] (TentaGel-S $NH_2$[11]) using ben-



a : $CH_3CO_2Na$, 190°C, b : $H_2$, 5% Pd-C, c : PPA, 145 °C, d : $AlCl_3$, benzene, e : *tert*-BuOK, $BrCH_2CO_2Et$, DMF, f : NaOH, dioxane-$H_2O$, g : $NaBH_4$, isopropyl alcohol, h : Fmoc-$NH_2$, PTS, DMF i : TGS-$NH_2$, PyBOP- HOBT-DIPEA, NMP

Chart 1

zotriazole-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP)–1-hydroxylbenzotriazole (HOBT)[12] as the coupling reagent. In order to assess the relative rate of cleavage of **11** and **12**,[6] Fmoc-Val derivatives prepared from **11** and **12** were treated with 50% trifluoroacetic acid (TFA)–5% phenol in dichloromethane (DCM) as the cleavage cocktail for specific time intervals. In this way, the approximate half lives were *ca.* 12 min for **11** and *ca.* 3 min for **12**. These results indicated that the Fmoc-Val derivative from **12** can be rapidly cleaved at a lower concentration of TFA than that for **11**. The rupture of the amino carbon–nitrogen linkage was significantly affected depending on the carbon chain spacer between the 10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cycloheptene moiety and the polymeric support.

## Experimental

Melting points were determined on a Shibayama micro-melting point apparatus and are uncorrected. $^1$H-NMR spectra were recorded at 270 MHz on a JEOL JNM EX 270 instrument. Tetramethylsilane (TMS) was used as an internal standard. Elemental analysis was performed in the Microanalysis Service Center, Faculty of Pharmaceutical Sciences, Kyoto University. Flash column chromatography was carried out on Silica gel 60 (Nakarai tesque, 230—400 mesh). The following abbreviations are used: DIPEA, diisopropylethylamine; NMP, 1-methyl- 2-pyrrolidinone. NMM; 4-methylmorpholine.

***m*-Methoxystilbene-2-carboxylic Acid (3)** A mixture of 2-carboxybenzaldehyde (**1**) (5.0 g, 33.3 mmol), *m*-methoxyphenylacetic acid (**2**) (5.53 g, 33.3 mmol) and sodium acetate (5.46 g, 66.6 mmol) were heated with mechanical stirring at 190—195 °C for 8 h. The evolved carbon dioxide was trapped with 10% $Ba(OH)_2$ solution. After cooling, the residue was dissolved in AcOEt and water. The AcOEt solution was separated, and the water layer was acidified with 10% HCl (pH about 5) and extracted with AcOEt. The combined AcOEt solution was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give crude product that was purified by silica gel column chromatography with AcOEt–hexane (1 : 4) as eluent to give **3** (6.40 g, 76% ). The product was recrystallized from AcOEt–hexane. Colorless needles, mp 141—144 °C. *Anal.* Calcd for $C_{16}H_{14}O_3$: C, 75.58; H, 5.55. Found: C, 75.42; H, 5.29. $^1$H-NMR ($CDCl_3$ ) $\delta$: 3.83 (s, 3H, $OCH_3$), 6.84 (dq, 1H, $J$=8.1, 2.7, 0.8 Hz, C4′-H), 7.02 (d, 1H, $J$=15.9 Hz, *trans* olefin, $-CH_A=C\underline{H}_B-$), 7.09 (dd, 1H, $J$=2.7, 1.6 Hz, C2′-H), 7.16 (dq, 1H, $J$=8.1, 1.6, 0.8 Hz, C6′-H), 7.28 (t, 1H, $J$=8.1 Hz, C5′-H), 7.36 (dt, 1H, $J$=7.6 (t), 1.1 (d) Hz, C4-H), 7.57 (dt, 1H, $J$=7.6 (t), 1.1 (d) Hz, C5-H), 7.74 (dd, 1H, $J$=7.6, 1.1 Hz, C6-H), 8.06 (d, 1H, $J$=15.9 Hz, *trans* olefin,$-C\underline{H}_A=CH_B-$), 8.09 (dd, 1H, $J$=7.6, 1.1 Hz, C3-H).

**2-(*m*-Methoxyphenethyl)benzoic Acid (4)** A mixture of *m*-methoxystilbene-2-carboxylic acid (**3**) (3.0 g, 11.8 mmol) in methanol (30 ml) and 5% Pd–C (0.3 g) was stirred under an $H_2$ atmosphere for 1.5 h. The mixture was filtered, and the solution evaporated to give a colorless solid that was recrystallized from AcOEt–hexane to give colorless needles of **4** (2.69 g, 89%), mp 118—119 °C. *Anal.* Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.99; H, 6.46. $^1$H-NMR ($CDCl_3$) $\delta$: 2.92 (2H, t, $J$=7.8 Hz, $CH_2$), 2.34 (2H, t, $J$=7.8 Hz, $CH_2$), 3.78 (3H, s, $OCH_3$), 6.74 (1H, dd, $J$=7.8, 1.2 Hz, C4′-H), 6.78 (1H, t, $J$=1.2 Hz, C2′-H), 6.83 (1H, d, $J$=7.8 Hz, C6′-H), 7.19 (1H, t, $J$=7.8 Hz, C5′-H), 7.25 (1H, dd, $J$=7.5, 1.2 Hz, C6-H), 7.32 (1H, dt, $J$=7.5 (t), 1.2 (d) Hz, C4-H), 7.47 (1H, dt, $J$=7.5 (t), 1.5 (d) Hz, C5-H), 8.08 (1H, dd, $J$=7.5, 1.2 Hz, C3-H).

**Ethyl 2-[(5-Oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-yl)-oxy]acetate (7)** The starting material, 2-hydroxy-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (**6**), was prepared from **4** according to a previous paper.[6] To a solution of **6** (6.0 g, 26.8 mmol) in DMF (10 ml) was added *tert*-BuOK (3.31 g, 29.5 mmol) at 0 °C under $N_2$ atmosphere. The reaction mixture was stirred at room temperature for 5 min, ethyl bromoacetate (4.92 g, 29.5 mmol) was added, and the resulting solution heated at 90—100 °C with stirring for 4 h. Solvent was then removed at 80 °C under reduced pressure, the residue was dissolved in AcOEt, washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give crude product. The crude product was recrystallized from AcOEt–hexane to give **7** (7.10 g, 85%). Colorless plates, mp 72—73 °C. *Anal.* Calcd for

$C_{19}H_{18}O_4$: C, 73.53; H, 5.85. Found: C,73.25; H, 5.84. $^1$H-NMR ($CDCl_3$) $\delta$: 1.30 (t, 3H, $J=7.2$ Hz, $C\underline{H}_3CH_2$), 3.17 (s, 4H, $CH_2CH_2$), 4.28 (q, 2H, $J=7.2$ Hz, $CH_3C\underline{H}_2$), 4.67 (s, 2H, $CH_2$), 6.74 (d, 1H, $J=2.7$ Hz, C1-H), 6.83 (dd, 1H, $J=8.8$, 2.7 Hz, C3-H), 7.21 (dd, 1H, $J=7.3$, 1.3 Hz, C9-H), 7.32 (dt, 1H, $J=7.3$ (t), 1.3 (d ) Hz, C7-H), 7.42 (dt, 1H, $J=7.3$ (t), 1.3 (d) Hz, C8-H), 7.99 (dd, 1H, $J=7.3$, 1.3 Hz, C6-H), 8.14 (d, 1H, $J=8.8$ Hz, C4-H).

**2-[(5-Oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-yl)oxy]acetic Acid (8)** Ethyl 2-[(5-oxo-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-yl)oxy]acetate (7) (7.0 g, 22.6 mmol) was dissolved in 2 N NaOH (30 ml) and dioxane (30 ml) and the mixture stirred at room temperature for 4 h. The solution was then acidified with 10% HCl, and evaporated to dryness. The residue was dissolved in AcOEt, washed with brine, dried over anhydrous $MgSO_4$ and evaporated to give crude product. The crude product was recrystallized from AcOEt–hexane to give **8** (6.07 g, 95%). Colorless prisms, mp 167—168 °C. *Anal.* Calcd for $C_{17}H_{14}O_4$; C,72.33; H, 5.00. Found: C, 72.52; H, 5.09. $^1$H-NMR ($CDCl_3$) $\delta$: 3.18 (4H, s, $CH_2CH_2$), 4.74 (s, 2H, $CH_2$), 6.76 (d, 1H, $J=2.7$ Hz, C1-H), 6.86 (dd, $J=8.8$, 2.7 Hz, C3-H), 7.22 (dd, 1H, $J=7.6$, 1.3 Hz, C9-H), 7.32 (dt, 1H, $J=7.3$ (t), 1.3( d) Hz, C7-H), 7.43 (dt, 1H, $J=7.3$ (t), 1.3 (d) Hz, C8-H), 8.00 (dd, 1H, $J=7.3$, 1.3 Hz, C6-H), 8.15 (d, 1H, $J$ =8.8 Hz, C4-H).

**2-{[(*R*,*S*)-5-(Fmoc-amino)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-yl]oxy}acetic Acid (10)** To a solution of 2-[(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-2-yl)oxy]acetic acid (**8**) (0.3 g, 1.06 mmol) in isopropyl alcohol (10 ml) was added triethylamine (TEA) (141 $\mu$l, 1.06 mmol) and $NaBH_4$ (0.12 g, 3.18 mmol), and the mixture refluxed for 3 h. After solvent evaporation, the residue was dissolved in AcOEt and water, and the mixture acidified to about pH 4.0 with 10% HCl, and extracted with AcOEt. The solution was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give the unstable crude alcohol (**9**), which was immediately dissolved in DMF (10 ml). Fmoc-$NH_2$ (0.28 g, 1.17 mmol) and a catalytic amount of PTS were then added to the DMF solution and the reaction mixture stirred at room temperature for 16 h, and heated at 80 °C for 1.5 h with stirring. The DMF solution was diluted with water, and extracted with AcOEt. The solution was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give crude product that was recrystallized from MeOH to give **10** (0.47 g, 87%). Colorless amorphous powder, mp 214—218 °C (dec.). *Anal.* Calcd for $C_{32}H_{27}O_5N$: C, 5.38; H, 76.02; N, 2.77. Found: C, 75.67; H, 5.39; N, 2.82. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.90—3.27 (m, 4H, $CH_2CH_2$), 4.18—4.32 (m, 3H, $CO_2C\underline{H}_2C\underline{H}$), 4.62 ( br s, 2H, $OCH_2CO$), 6.02 (d, 1H, $J=7.8$ Hz, C5-H), 6.71 (br s, 2H, C1-H, C3-H), 7.08—7.94 (m, 13H, aromatic H), 8.55 (d, 1H, $J=7.8$ Hz, NH), 12.0—13.5 (br s, 1H, $CO_2H$).

**Loading of the Linker (10) onto the Amino Group of Polyethylene Glycol-Polystyrene Grafts Copolymer (TentaGel S $NH_2$)** To a solution of **10** (0.11 g, 0.22 mmol) in NMP (2 ml) was added TentaGel S $NH_2$ (0.25 g, 0.29 meq./g), PyBOP (0.113 g, 0.22 mmol) and HOBT (0.033 g, 0.22 mmol). The mixture was shaken to dissolve reagnts, and DIPEA (38 $\mu$l, 0.22 mmol) was then added to this mixture. The whole mixture was shaken at room temperature for 4 h. The resin was filtered, washed with DMF, DCM, MeOH and *tert*-butyl ethyl ether, and dried over $P_2O_5$. The resin (*ca.* 10 mg) was quantified by a spectrophotomeric method,[13] which indicated 97% incorporation.

**Cleavage Efficiency of Fmoc-Val Linker-Resin** The linker-resin (**11**) (0.138 g, 0.04 mmol) in DMF (1 ml) was manually coupled to Fmoc-Val (0.136 g, 0.4 mmol) with PyBOP (0.208 g, 0.4 mmol), HOBT (0.061 g, 0.4 mmol) and NMM (44 $\mu$l, 0.4 mmol) as coupling reagents for 4 h. The resin was washed with DMF, DCM, MeOH and *tert*-butyl ethyl ether, and dried over $P_2O_5$. The linker-resin (**12**) was coupled to Fmoc-Val in the same manner as above. Aliquots (*ca.* 10 mg) of the Fmoc-Val linker-resins were treated with 50% TFA/5% phenol in DCM. At regular time intervals, the mixture was quickly diluted with DMF, and the resins were filtered, washed with DMF, DCM, MeOH and *tert*-butyl ethyl ether, and dried over $P_2O_5$. The remaining Fmoc group on these resins were quantified by a spectrophotomeric method.

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