Development of Supramolecular Organo-Gel Based on Tripeptide Skeletons

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Boc-Ser-Val-Gly-OCH_2Ph (31) showed high gelation abilities in the aromatic solvents, particularly in toluene. The minimum gelation concentration of 31 in toluene was 10 mg/ml, suggesting that 2500 molecules of toluene were immobilized by each molecule of the tripeptide 31. The FT-IR data indicated that formation of antiparallel β-sheets through intermolecular hydrogen bonding was central to the generation of nanofibers during gelation.

Key words supramolecular gel; Boc-Ser-Val-Gly-OCH_2Ph; nanofiber

Low molecular weight (supramolecular) gels have attracted attention in the fields of supramolecular chemistry and material chemistry over the past two decades.[1—6] The gel phase is a wettable viscoelastic physical state that shares some properties of both liquid and solid phases. Gelation occurs when self-organizing gelators produce an entangled three-dimensional network of fibers that constrains the mobility of the embedded solvent molecules. Low molecular weight gelators based on amino acids,[7—10] ureas,[11—13] amides,[14—16] and saccharides[17—19] have been developed, and the responses of these gels to photo irradiation,[20,21] ultrasonic frequencies,[22,23] and biologically active agents[10,24,25] have been described. However, organic reactions (covalent bond forming reactions) in the gel phase have received relatively little attention.[26,27] We are trying to develop novel organic reactions in a supramolecular gel phase using a dual-functional molecule that is capable of catalyzing reaction as well as forming gel. In this paper, we describe the synthesis and gelation properties of several tripeptide derivatives, which form the backbone of the dual-functional molecule.

N-t-Butoxycarbonyl (Boc) and *O*-benzyl protected tripeptide gelators, composed of Gly, Ala, and Val, were designed because this combination was previously and accidentally shown to yield interesting gelation properties.[28] Twenty-seven tripeptides, **1**—**27**, based on various combinations of Gly, Ala, and Val, were synthesized by repeated condensation and deprotection in the liquid phase (Chart 1).[29—31] Each amino acid benzyl ester (third fragment) was reacted with a Boc-protected amino acid (second fragment) under 1-hydroxybenzotriazole hydrate ($HOBt \cdot H_2O$)/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC·HCl)/triethylamine conditions in 27% to quantitative yields. The dipeptide was subsequently deprotected by removal of the Boc group in HCl/dioxane to give an *O*-benzyl dipeptide. A Boc-protected amino acid (first fragment) was then introduced to the dipeptide under similar conditions to afford the desired tripeptides **1**—**27** in yields of 15—99%.

The gelation abilities of the 27 tripeptide derivatives in each of ten organic solvents (hexane, EtOAc, $CHCl_3$, CH_2Cl_2, acetone, acetonitrile, EtOH, MeOH, toluene, and chlorobenzene) were surveyed as follows. Each tripeptide/solvent combination was mixed in a microtube and (as appropriate) irradiated with ultrasound for 30 min at temperatures ranging from room temperature to 60 °C to afford a homogeneous solution. The solutions were stored in an incubator at 20 °C for 24 h. When the tube was inverted and tapped, the absence of a fluid phase was defined as a gel (transparent or opaque gel), and a crumpled jelly object was defined as a partial gel. Table 1 shows the results of the first screening and the minimum gelation concentration for those tripeptide/solvent combinations that yielded a gel.

The results shown in Table 1 indicate that toluene and chlorobenzene, which have aromatic rings, showed the highest propensity for gelation among the solvents assayed, suggesting that a π–π interaction between the aromatic rings of the solvent and the benzyl group of the tripeptide played a crucial role in gelation. The tripeptide sequences X-Val-Gly **7**—**9** and X-Ala-Ala **13**—**15** displayed inherently high gelation abilities for these solvents.

A new series of tripeptides with the sequences X-Val-Gly and X-Ala-Ala were synthesized and examined in detail. For the *N*-terminal amino acid (X), serine and glutamine were selected because of their hydrogen bonding abilities, and phenylalanine was selected for its ability to engage in π–π stacking interactions. Tripeptides **28**—**33** were synthesized *via* the route described in Chart 1. The results from a second gelation screening assay, using the additional solvents *o*-, *m*-, and *p*-xylenes as aromatic solvents in addition to the solvents assayed in the first screening, are summarized in Table 2.

Among these tripeptides, Boc-Ser-Val-Gly-OCH_2Ph (**31**) showed excellent gelation properties in the aromatic solvents, particularly in toluene (Fig. 1a), most likely because of the

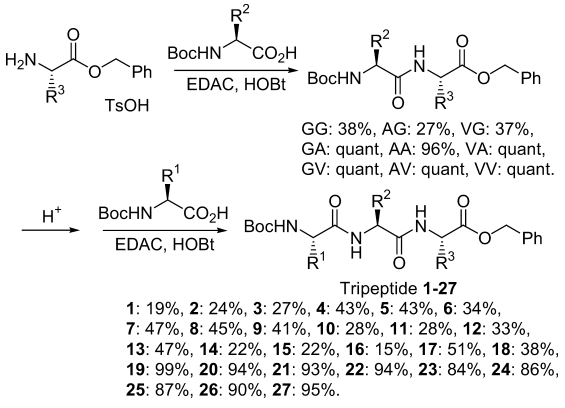

Chart 1. Synthetic Route for Tripeptides **1**—**27**

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Table 1. Gelation Studies of Tripeptides **1**—**27** in Various Organic Solvents

Compd. No.	Boc-XXX-OCH_2Ph	Hexane	EtOAc	$CHCl_3$	CH_2Cl_2	Acetone	CH_3CN	EtOH	MeOH	Toluene	Cl–C_6H_5
1	G-G-G	*i*	*p*	*h*	*h*	*h*	*h*	*h*	*h*	*i*	*i*
2	A-G-G	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	O (100)	T (100)
3	V-G-G	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*
4	G-A-G	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*
5	A-A-G	*i*	*p*	*h*	*h*	*h*	*h*	*h*	*p*	*i*	*i*
6	V-A-G	*i*	*h*	*h*	*h*	*h*	*i*	*h*	*h*	O (50)	T (33)
7	G-V-G	*i*	PG	*p*	*p*	*i*	*i*	PG	PG	O (33)	O (66)
8	A-V-G	*i*	O (100)	*h*	*h*	*p*	O (100)	O (100)	*h*	PG	O (33)
9	V-V-G	*i*	O (50)	*h*	*h*	*p*	O (100)	PG	*h*	PG	T (33)
10	G-G-A	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*
11	A-G-A	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	PG	PG
12	V-G-A	*i*	*h*	*h*	*h*	*h*	*i*	*h*	*h*	*p*	*h*
13	G-A-A	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	O (33)	T (66)
14	A-A-A	*i*	*h*	*h*	*h*	*h*	O (100)	*h*	*h*	O (50)	O (50)
15	V-A-A	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	O (100)	O (33)
16	G-V-A	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*i*	PG
17	A-V-A	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*i*	PG
18	V-V-A	*i*	O (100)	*h*	*h*	PG	PG	PG	*h*	PG	T (50)
19	G-G-V	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*i*	*i*
20	A-G-V	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*
21	V-G-V	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*
22	G-A-V	PG	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*i*	*p*
23	A-A-V	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	PG	*h*
24	V-A-V	O (33)	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*
25	G-V-V	PG	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*
26	A-V-V	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*h*
27	V-V-V	*i*	*h*	*h*	*h*	*h*	O (100)	*h*	*h*	*h*	*i*

The concentration of the tripeptide was up to 100 mg/ml. The minimum gelation concentration (mg/ml) is shown in a parenthesis. *h*: homogeneous solution; T: transparent gel; O: opaque gel; PG: partial gel; *p*: precipitation; *i*: insoluble.

Table 2. Gelation Studies of Tripeptides **28**—**33** in Various Organic Solvents

Compd. No.	Boc-XXX-OCH_2Ph	Hexane	EtOAc	$CHCl_3$	CH_2Cl_2	Acetone	CH_3CN	EtOH	MeOH	Toluene	Cl–C_6H_5	*o*-Xylene	*m*-Xylene	*p*-Xylene
28	S-A-A	*i*	*i*	*h*	*h*	*i*	*h*	*h*	*h*	*i*	*i*	*i*	*i*	*i*
29	F-A-A	*i*	*h*	*h*	*h*	*i*	*h*	*h*	*h*	O (50)	O (33)	*i*	*i*	*i*
30	Q-A-A	*i*	*i*	*h*	*i*	O (100)	*i*	*h*	*h*	*i*	*i*	*i*	*i*	*i*
31	S-V-G	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	O (10)	T (33)	O (33)	O (33)	O (33)
32	F-V-G	*i*	*h*	*h*	*h*	*h*	*h*	*h*	*h*	*i*	O (33)	*i*	*i*	*i*
33	Q-V-G	*i*	*i*	*i*	*i*	*i*	*i*	*i*	*i*	*i*	*p*	*i*	*i*	*i*

The concentration of the tripeptide was up to 100 mg/ml. The minimum gelation concentration (mg/ml) is shown in a parenthesis. *h*: homogeneous solution; T: transparent gel; O: opaque gel; PG: partial gel; *p*: precipitation; *i*: insoluble.

additional hydrogen bonding capacity introduced by the hydroxy group of serine. The minimum gelation concentration of **31** in toluene was 10 mg/ml, suggesting that 2500 molecules of toluene were immobilized by each molecule of the tripeptide **31**.

Boc-Ser-Val-Gly-OCH_2Ph (**31**) yielded the best gelation properties, and the morphological characteristics of its toluene gel were observed by scanning electron microscopy (SEM), three-dimensional (3D) laser scanning optical microscopy and digital optical microscopy (Fig. 1). SEM revealed a plate-like microcrystalline structure (1 μm in width, Fig. 1b). 3D laser microscopy revealed a network of entangled nanofibers, the minimum height of which was estimated to be 60—70 nm (Fig. 1c). The imaging conditions were responsible for the observation of two morphologies, microcrystals or nanofibers, for the toluene gel of **31**. 3D optical imaging permitted observation of the fibers under wet conditions (in the presence of solvent). The SEM measurement required solvent removal under reduced pressure, which induced the nanofibers to form a microcrystalline phase. 3D optical imaging was used to follow the nanofiber-to-microcrystal transition (Fig. 1d). A digital optical microscope, in which the gel remained wet, also revealed long fibers (Fig. 1e).

The intermolecular interactions involved in stabilizing the toluene gel of **31** were characterized by ^{1}H-NMR. The short transversal relaxation time in the gel state prevented collection of the gel state NMR spectrum of gelator **31**. We therefore inferred the intermolecular interactions and driving force for gelation of **31** by measuring the solution-phase NMR spectrum as a function of temperature and concentration of **31** (Fig. 2). At 40 °C, 5 mg/ml **31** in toluene *d*-8, three signals corresponding to the NH protons (Ha, Hb, Hc indicated in the structure of Fig. 2) were observed at δ 7.18 (Hb), 6.69 (Hc), and 5.67 (Ha), respectively (all shifts are given in ppm). As the concentration of **31** increased and/or the tem-

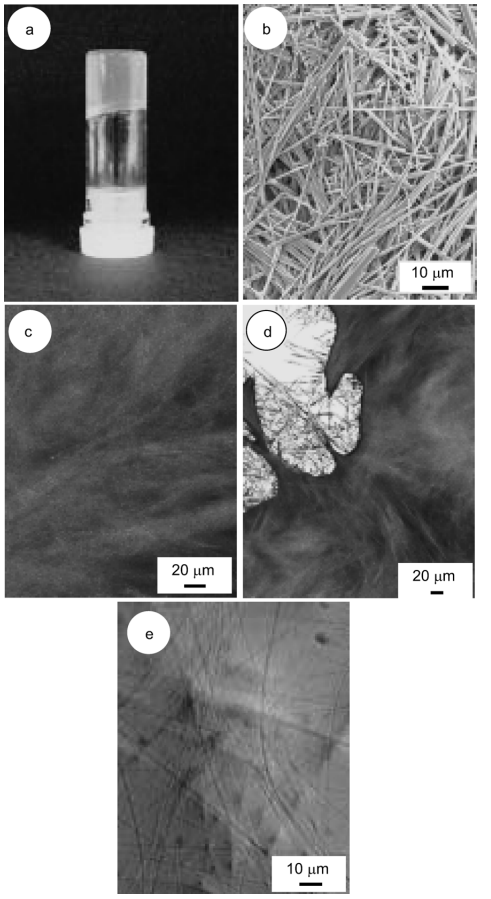

Fig. 1. The Morphologies of the Toluene Gel of **31**

(a) A photograph of a toluene gel of **31** (10 mg/ml). (b) SEM image of microcrystals of **31**, formed during sample drying. (c, d) 3D scanning laser images of the toluene gel (wet state). (e) Digital microscopy of the toluene gel (wet state).

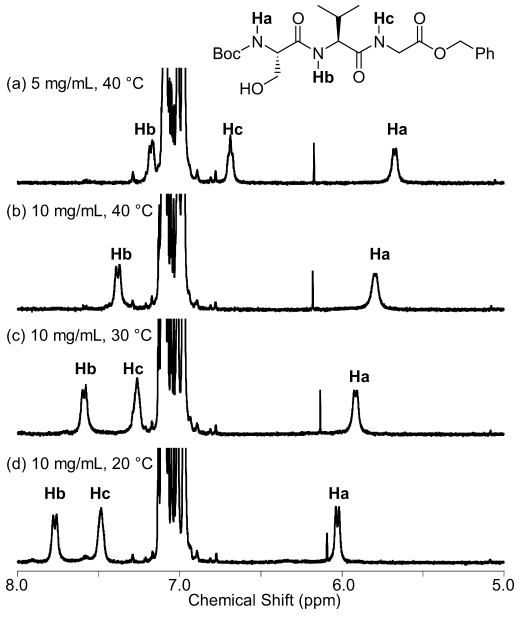

Fig. 2. ^{1}H-NMR (400 MHz) Spectra of Tripeptide **31** in Toluene *d*-8

perature was reduced, the chemical shifts of the amidic NH protons shifted dramatically downfield. This behavior was ascribed to the formation of hydrogen bonds between these amidic protons and the carbonyl oxygen. An increase in the

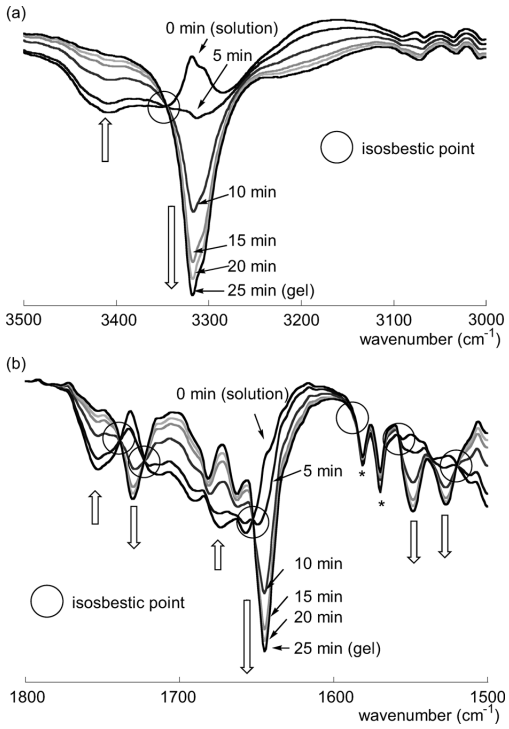

Fig. 3. Time Course IR Spectra of **31** during the Phase Transition from a Solution to a Gel

10 mg/ml **31** in toluene *d*-8, NaCl liquid cell (path length: 0.1 mm), * denotes an absorption peak from the toluene *d*-8.

hydrogen bonding network and gelation, therefore, appeared to be closely related.

The solution-to-gel phase transition was tracked by FT-IR using a liquid cell with toluene *d*-8 (Fig. 3).

The time course IR spectra of **31** at 3200—3450 cm^{-1} (in the region of amide A) is shown in Fig. 3a. As the solution phase transitioned to the gel phase, the strength of the free NH stretch at 3410 cm^{-1} decreased and the strength of the hydrogen bonding NH stretch (3319 cm^{-1}) increased with an isosbestic point. At 1500—1800 cm^{-1} (amide I and II regions, Fig. 3b), the strength of the free carbonyl stretch at 1678 cm^{-1} decreased with a concomitant increase in the strength of the hydrogen bonding carbonyl stretch at 1645 cm^{-1}, with several isosbestic points. Yamada and coworkers reported that parallel or antiparallel β-sheet secondary structures of (tri-)peptides could be distinguished by the presence or absence, respectively, of the characteristic IR absorption at 1690 cm^{-1} in addition to the absorptions at 1630 cm^{-1} and 1527 cm^{-1}, independent of the sequence.[32,33] Taking into consideration of their data, in the solution phase, tripeptide **31** absorbed at 3410, 1678, and 1495 cm^{-1}, indicating the absence of specific secondary structure. In contrast, the gel phase showed absorptions at 3318, 1681, 1645, and 1527 cm^{-1}, suggesting the formation of antiparallel β sheets.

In conclusion, we synthesized 27 tripeptides (combinations of Gly, Ala, and Val) for a first screening assay, and 6 tripeptides for a second screening assay, to test for gelation in organic solvents. We identified Boc-Ser-Val-Gly-OCH_2Ph (**31**) as an excellent gelator for aromatic solvents. Boc-Ser-Val-Gly-OCH_2Ph (**31**) yielded particularly high gelation abilities in toluene, with a minimum gelation concentration of 10 mg/ml. The FT-IR data suggested that formation of an-

tiparallel β-sheets through intermolecular hydrogen bonding was central to the generation of nanofibers during gelation. Identification and characterization of the gelation properties of **31** lay the foundation for developing a dual-functional molecule that displays both catalytic activity and gelation ability.

Experimental

Tripeptides **1**—**33** were synthesized by the procedure described in the text.[29—31] FT-IR spectra of **31** were collected on a JASCO FT/IR-4200, using a NaCl liquid cell (0.1 mm path length) with toluene *d*-8. SEM, 3D scanning laser microscopy, and digital optical microscopy were performed using a VE-9800 SEM, a VK-9700 Generation II Color Laser Scanning Microscope, and a VHX-1000 (KEYENCE) digital optical microscope, respectively.

Characterization Data of New Compounds Boc-Val-Gly-Gly-OCH_2Ph (**3**): Pale yellow viscous oil; IR (neat) 3307, 3067, 2968, 2933, 1751, 1656, 1526, 1366, 1174, 1032, 752 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.32—7.33 (m, 5H), 7.06 (br, 1H), 6.82 (br, 1H), 5.17 (s, 2H), 5.07 (br, 1H), 4.16—3.95 (m, 4H), 3.87 (dd, J=6.8 Hz, J=6.8 Hz, 1H), 2.19—2.11 (m, 1H), 1.41 (s, 9H), 0.98 (d, J=6.8 Hz, 3H), 0.94 (d, J=6.8 Hz, 3H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 172.5, 169.4, 156.1, 135.0, 128.5, 128.3, 128.2, 80.2, 67.1, 60.6, 42.9, 41.3, 30.6, 28.3, 19.3, 18.1; MS (EI) *m/z* (rel. intensity) 421 [M^+, (2)], 365 (5), 201 (14), 172 (75), 116 (100), 72 (86), 57 (34); HR-MS (EI) Calcd for $C_{21}H_{31}O_6N_3$ (M^+) 421.2213, Found 421.2207.

Boc-Val-Ala-Gly-OCH_2Ph (**6**): Clear viscous oil; IR (KBr) 3299, 3068, 2975, 1756, 1642, 1528, 1391, 1173, 1019, 698, 419 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.38—7.30 (m, 5H), 6.78 (br, 1H), 6.49 (br, 1H), 5.17 (s, 2H), 5.00 (br, 1H), 4.55 (qd, J=7.3 Hz, J=7.3 Hz, 1H), 4.05 (d, J=5.4 Hz), 3.91 (dd, J=6.2 Hz, J=6.2 Hz, 1H), 2.21—2.07 (m, 1H), 1.43 (s, 9H), 1.40 (d, J=7.3 Hz, 3H), 0.96 (d, J=7.0 Hz, 3H), 0.91 (d, J=6.8 Hz, 3H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 180.7, 172.2, 171.5, 169.3, 155.9, 135.0, 128.5, 128.4, 128.2, 80.0, 67.1, 60.0, 48.7, 41.3, 31.0, 28.3, 19.3, 18.3, 17.8; MS (EI) *m/z* (rel. intensity) 435 [M^+, (2)], 365 (5), 243 (14), 172 (53), 116 (100), 91 (76), 72 (78), 57 (32); HR-MS (EI) Calcd for $C_{22}H_{33}O_6N_3$ (M^+) 435.2369, Found 435.2371.

Boc-Gly-Gly-Ala-OCH_2Ph (**10**): Viscous oil; IR (neat) 3307, 3068, 2979, 2937, 1661, 1528, 1367, 1251, 1162, 751, 698 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.25—7.34 (m, 5H), 6.86—6.76 (br, 1H), 6.75—6.65 (br, 1H), 5.18—5.10 (br, 1H), 5.17 (ABq, 8.5 Hz, J_{AB}=12.2 Hz, 2H), 4.61 (qd, J=7.0 Hz, J=7.0 Hz, 1H), 3.99 (ABC, 32.4 Hz, J=16.7 Hz, J=5.4 Hz, 2H), 3.82 (d, J=5.7 Hz, 2H), 1.45 (s, 9H), 1.43 (d, J=7.0 Hz, 3H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 172.3, 170.3 168.7, 156.1, 135.1, 128.3, 128.1, 127.7, 79.9, 66.9, 48.2, 44.0, 42.6, 20.2, 17.5; MS (FAB+, Glycerol) *m/z* (rel. intensity) 435 [$(M+Glycerol+H)^+$, (1)], 394 [$(M+H)^+$, 8], 338 (12), 229 (40), 173 (62), 91 (67), 72 (100), 57 (21); HR-MS (EI) Calcd for $C_{19}H_{28}O_6N_3$ $(M+H)^+$ 394.1978, Found 394.1979.

Boc-Val-Gly-Ala-OCH_2Ph (**12**): White solid; mp 107—109 °C; IR (KBr) 3341, 2966, 1742, 1690, 1644, 1523, 1390, 1247, 1166, 1047, 753, 652 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.36—7.38 (m, 5H), 7.00 (br, 1H), 6.92 (br, 1H), 5.16 (ABq, 9.0 Hz, J_{AB}=12.4 Hz, 2H), 5.13 (br, 1H), 4.61 (qd, J=7.3 Hz, J=7.3 Hz, 1H), 4.15—4.07 (m, 1H), 3.98—3.87 (m, 1H), 1.43 (s, 9H), 1.42 (d, J=4.9 Hz, 3H), 0.98—0.91 (m, 6H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 172.4, 172.3, 168.5, 155.8, 135.2, 128.4, 128.2, 127.9, 79.9, 67.1, 60.2, 48.3, 43.0, 30.8, 28.3, 19.3, 17.9; MS (EI) *m/z* (rel. intensity) 435 [M^+, (2)], 379 (8), 257 (11), 172 (54), 116 (100), 91 (78), 72 (79), 57 (38); HR-MS (EI) Calcd for $C_{22}H_{33}O_6N_3$ (M^+) 435.2370, Found 435.2372.

Boc-Gly-Val-Ala-OCH_2Ph (**16**): Viscous oil; IR (KBr) 3296, 2973, 1748, 1705, 1640, 1543, 1367, 1163, 1050, 750, 695 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.30—7.36 (m, 5H), 6.88 (d, J=8.9 Hz, 1H), 6.75 (d, J=7.3Hz, 1H), 5.31—5.23 (m, 1H), 5.16 (ABq, 13.0 Hz, J_{AB}=12.2 Hz, 2H), 4.60 (qd, J=7.3 Hz, J=7.3 Hz, 1H), 4.30 (dd, J=8.9 Hz, J=8.9 Hz, 1H), 3.81 (ABC, 23.0 Hz, J_{AB}=17.0 Hz, J=5.4 Hz, 2H), 2.19—2.02 (m, 1H), 1.44 (s, 9H), 1.42 (d, J=7.3 Hz, 3H), 0.93 (d, J=6.8 Hz, 3H), 0.91 (d, J=7.0 Hz, 3H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 172.1, 171.0, 169.8, 155.9, 135.1, 128.3, 128.1, 128.0, 79.8, 66.9, 58.2, 48.1, 44.0, 31.3, 28.2, 19.0, 18.2, 17.6; MS (EI) *m/z* (rel. intensity) 435 [M^+, (1)], 379 (4), 257 (12), 229 (40), 173 (62), 91 (67), 72 (100), 57 (21); HR-MS (EI) Calcd for $C_{22}H_{33}O_6N_3$ (M^+) 435.2369, Found 435.2366.

Boc-Val-Val-Ala-OCH_2Ph (**18**): White solid; mp 171—173 °C; IR (KBr) 3281, 2962, 1742, 1688, 1647, 1526, 1368, 1247, 1173, 744, 698 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.32—7.35 (m, 5H), 6.47—6.62 (br, 2H), 5.17 (ABq, 12.7 Hz, J_{AB}=12.2 Hz, 2H), 5.03 (br, 1H), 4.61 (qd, J=7.3 Hz, J=7.3 Hz, 1H), 4.26 (dd, J=8.4 Hz, J=6.8 Hz, 1H), 3.91 (dd, J=6.5 Hz, J=6.5 Hz, 1H), 2.19—2.15 (m, 2H), 1.45 (s, 9H), 1.41 (d, J=7.3 Hz, 3H), 0.97—0.90 (m, 12H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 172.2, 172.0, 170.7, 155.8, 135.2, 128.4, 128.3, 128.1, 80.8, 67.1, 60.2, 58.4, 48.1, 31.3, 30.8, 28.3, 19.3, 19.2, 18.2, 18.0, 17.5; MS (EI) *m/z* (rel. intensity) 477 [M^+, (8)], 404 (5), 299 (11), 271 (36), 200 (36), 116 (67), 72 (100), 57 (22); HR-MS (EI) Calcd for $C_{25}H_{39}O_6N_3$ (M^+) 435.2839, Found 477.2847.

Boc-Val-Ala-Val-OCH_2Ph (**24**): Viscous oil; IR (KBr) 3292, 2968, 1734, 1714, 1645, 1528, 1391, 1367, 1166, 1018, 752, 698 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.36—7.30 (m, 5H), 6.87—6.75 (br, 2H), 5.21 (br, 1H), 5.17 (ABq, 23.1 Hz, J_{AB}=12.4 Hz, 2H), 4.60—4.53 (m, 2H), 3.95 (br, 1H), 2.24—2.05 (m, 2H), 1.44 (s, 9H), 1.36 (d, J=6.8 Hz, 3H), 0.98—0.84 (m, 12H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 172.0, 171.6, 171.3, 155.7, 135.2, 128.4, 128.3, 128.2, 80.0, 67.0, 57.3, 48.8, 31.2, 31.0, 28.3, 19.3, 19.0, 18.2, 17.7, 17.5; MS (EI) *m/z* (rel. intensity) 477 [M^+, (9)], 342 (13), 305 (13), 271 (20), 215 (25), 172 (37), 116 (97), 91 (76), 72 (100), 57 (29); HR-MS (EI) Calcd for $C_{25}H_{39}O_6N_3$ (M^+) 447.2839, Found 477.2837.

Boc-Ala-Val-Val-OCH_2Ph (**26**): Viscous oil; IR (KBr) 3299, 3069, 2969, 1742, 1714, 1648, 1549, 1391, 1367, 1248, 1170, 1048, 1026, 698 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.36—7.31 (m, 5H), 6.80—6.71 (br, 1H), 6.50—6.36 (br, 1H), 5.16 (ABq, 20.1 Hz, J_{AB}=12.2 Hz, 2H), 5.07—4.86 (br, 1H), 4.57 (dd, J=8.8 Hz, J=4.6 Hz, 1H), 4.24 (br, 1H), 4.22—4.10 (br, 1H), 2.18 (m, 2H), 1.44 (s, 9H), 1.34 (d, J=7.3 Hz, 3H), 0.94—0.84 (m, 12H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 172.8, 171.3, 171.2, 155.3, 135.2, 128.4, 128.2, 79.7, 66.9, 58.6, 57.0, 50.0, 31.1, 30.9, 28.3, 19.2, 19.0, 18.9, 18.3, 17.7; MS (EI) *m/z* (rel. intensity) 477 [M^+, (6)], 333 (9), 291 (8), 271 (11), 243 (21), 187 (22), 172 (15), 91 (50), 72 (100), 57 (14); HR-MS (EI) Calcd for $C_{25}H_{39}O_6N_3$ (M^+) 447.2838, Found 477.2831.

Boc-Ser-Ala-Ala-OCH_2Ph (**28**): White solid; mp 91 °C; IR (KBr) 3311, 3068, 2979, 2933, 1743, 1649, 1531, 1455, 1367, 1251, 1166, 1061, 752, 698 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.36—7.33 (m, 5H), 6.83 (br, 1H), 6.76 (br, 1H), 5.45 (br, 1H), 5.16 (ABq, 11.1 Hz, J_{AB}=12.2 Hz, 2H), 4.60 (ABC, 12.6 Hz, J=7.3 Hz, J=7.3 Hz, 1H), 4.47 (ABC, 12.6 Hz, J=7.3 Hz, J=7.3 Hz, 1H), 4.20 (br, 1H), 4.01 (m, 1H), 3.63 (m, 1H), 3.32 (br, 1H), 1.45 (s, 9H), 1.45—1.39 (m, 6H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 172.4, 171.9, 170.9, 155.6, 135.1, 128.4, 128.3, 128.0, 80.3, 67.1, 63.1, 55.4, 49.2, 48.3, 28.3, 18.0, 17.9; MS (EI) *m/z* (rel. intensity) 437 [M^+, (0.7)], 407 (30), 351 (19), 259 (15), 203 (22), 175 (32), 131 (31), 91 (100), 57 (41); HR-MS (EI) Calcd for $C_{21}H_{31}O_7N_3$ (M^+) 437.2162, Found 437.2167.

Boc-Gln-Ala-Ala-OCH_2Ph (**30**): White solid; mp 167—170 °C; IR (KBr) 3320, 3067, 2979, 1738, 1657, 1528, 1454, 1166, 1051, 698 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$+CD_3OD) δ: 7.38—7.33 (m, 5H), 5.16 (ABq, 11.5 Hz, J_{AB}=12.2 Hz, 2H), 4.51 (q, J=7.3 Hz, 1H), 4.37 (q, J=7.3 Hz, 1H), 4.10—4.03 (m, 1H), 2.28 (t, J=7.7 Hz, 2H), 2.06—1.90 (m, 2H), 1.44 (s, 9H), 1.42 (d, J=7.3 Hz, 3H), 1.36 (d, J=7.0 Hz, 3H); ^{13}C-NMR (68 MHz, $CDCl_3$+CD_3OD) δ: 175.8, 172.4, 172.1, 171.8, 155.7, 134.9, 128.1, 127.9, 127.6, 79.7, 66.7, 53.5, 48.5, 47.9, 31.1, 27.8, 17.3, 16.8; MS (EI) *m/z* (rel. intensity) 478 [M^+, (5)], 216 (30), 201 (55), 173 (30), 145 (40), 91 (100); HR-MS (EI) Calcd for $C_{23}H_{34}O_7N_4$ (M^+) 478.2428, Found 478.2437.

Boc-Ser-Val-Gly-OCH_2Ph (**31**): White solid; mp 113—114 °C; IR (KBr) 3310, 3072, 2970, 1746, 1649, 1527, 1367, 1173, 1062, 698 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.38—7.31 (m, 5H), 6.97 (br d, J=8.5 Hz, 1H), 6.88 (br, 1H), 5,54 (br d, J=6.5 Hz, 1H), 5.16 (s, 2H), 4.33 (dd, J=5.9 Hz, J=8.5 Hz, 1H), 4.21 (br, 1H), 4.09—4.03 (m, 2H), 4.00 (br, 1H), 3.70—3.60 (m, 1H), 3.36 (br, 1H), 2.32—2.25 (m, 1H), 1.44 (s, 9H), 1.02—0.88 (m, 6H); ^{13}C-NMR (68 MHz, $CDCl_3$) δ: 171.5, 169.7, 155.8, 134.9, 128.5, 128.4, 128.2, 80.4, 67.2, 63.0, 58.7, 55.3, 41.2, 30.2, 28.3, 19.3, 17.7; MS (EI) *m/z* (rel. intensity) 451 [M^+, (0.4)], 421 (28), 365 (12), 259 (19), 203 (43), 159 (39), 91 (91), 72 (100), 57 (30); HR-MS (EI) Calcd for $C_{22}H_{33}O_7N_3$ (M^+) 451.2318, Found 451.2325.

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References and Notes

1) Terech P., Weiss R. G., *Chem. Rev.*, **97**, 3133—3160 (1997).
2) Estroff L. A., Hamilton A. D., *Chem. Rev.*, **104**, 1201—1218 (2004).
3) George M., Weiss R. G., *Acc. Chem. Res.*, **39**, 489—497 (2006).
4) Dastidar P., *Chem. Soc. Rev.*, **37**, 2699—2715 (2008).

5) Suzuki M., Hanabusa K., *Chem. Soc. Rev.*, **38**, 967—975 (2009).
6) Cravotto G., Cintas P., *Chem. Soc. Rev.*, **38**, 2684—2697 (2009).
7) Menger F. M., Caran K. L., *J. Am. Chem. Soc.*, **122**, 11679—11691 (2000).
8) Ganesh S., Prakash S., Jayakumar R., *Biopolymers*, **70**, 346—354 (2003).
9) Maji S. K., Malik S., Drew M. G. B., Nandi A. K., Banerjee A., *Tetrahedron Lett.*, **44**, 4103—4107 (2003).
10) Zhang Y., Gu H., Yang Z., Xu B., *J. Am. Chem. Soc.*, **125**, 13680—13681 (2003).
11) Wang G., Hamilton A. D., *Chem. Commun.*, **2003**, 310—311 (2003).
12) Wang G., Hamilton A. D., *Chem. Eur. J.*, **8**, 1954—1961 (2002).
13) Yamanaka M., Fujii H., *J. Org. Chem.*, **74**, 5390—5394 (2009).
14) Hanabusa K., Yamada M., Kimura M., Shirai H., *Angew. Chem., Int. Ed. Engl.*, **35**, 1949—1951 (1996).
15) Tomioka K., Sumiyoshi T., Narui S., Nagaoka Y., Iida A., Miwa Y., Taga T., Nakano M., Handa T., *J. Am. Chem. Soc.*, **123**, 11817—11818 (2001).
16) Sumiyoshi T., Nishimura K., Nakano M., Handa T., Miwa Y., Tomioka K., *J. Am. Chem. Soc.*, **125**, 12137—12142 (2003).
17) Hamachi I., Kiyonaka S., Shinkai S., *Chem. Commun.*, **2000**, 1281—1282 (2000).
18) Hamachi I., Kiyonaka S., Shinkai S., *Tetrahedron Lett.*, **42**, 6141—6145 (2001).
19) Kiyonaka S., Shinkai S., Hamachi I., *Chem. Eur. J.*, **9**, 976—983 (2003).
20) Collier J. H., Hu B.-H., Ruberti J. W., Zhang J., Shum P., Thompson D. H., Messersmith P. B., *J. Am. Chem. Soc.*, **123**, 9463—9464 (2001).
21) Yagai S., Nakajima T., Kishikawa K., Kohmoto S., Karatsu T., Kitamura A., *J. Am. Chem. Soc.*, **127**, 11134—11139 (2005).
22) Naota T., Koori H., *J. Am. Chem. Soc.*, **127**, 9324—9325 (2005).
23) Isozaki K., Takaya H., Naota T., *Angew. Chem., Int. Ed.*, **46**, 2855—2857 (2007).
24) Kiyonaka S., Sada K., Yoshimura I., Shinkai S., Kato N., Hamachi I., *Nature Materials*, **3**, 58—64 (2003).
25) Wada A., Tamaru S., Ikeda M., Hamachi I., *J. Am. Chem. Soc.*, **131**, 5321—5330 (2009).
26) Miravet J. F., Escuder B., *Chem. Commun.*, **2005**, 5796—5798 (2005).
27) Rodriguez-Llansola F., Escuder B., Miravet J. F., *J. Am. Chem. Soc.*, **131**, 11478—11484 (2009).
28) Among the tripeptides **1**—**27**, galation of Boc-Ala-Val-Ala-OCH_2Ph (**17**) was reported. Das A. K., Bose P. P., Drew M. G. B., Banerjee A., *Tetraherdon*, **63**, 7432—7442 (2007).
29) Kiho T., Nakayama M., Yasuda K., Miyakoshi S., Inukai M., Kogen H., *Bioorg. Med. Chem.*, **12**, 337—361 (2004).
30) Kitamoto T., Marubayashi S., Yamazaki T., *Chem. Lett.*, **35**, 1264—1265 (2006).
31) Nozaki S., *J. Peptide Sci.*, **12**, 147—153 (2006).
32) Yamada N., Koyama E., Imai T., Matsubara K., Ishida S., *Chem. Commun.*, **1996**, 2297—2298 (1996).
33) Yamada N., Ariga K., Naito M., Matsubara K., Koyama E., *J. Am. Chem. Soc.*, **120**, 12192—12199 (1998).