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FT-IR and CD Measurement of *Z*-Dehydrophenylalanine-Containing Peptides in the Solid State and in Solution

Yoshihito INAI,† Yasuhiro SAKAKURA,* and Tadamichi HIRABAYASHI

Department of Environmental Technology and Urban Planning, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466–8555, Japan
**Department of Materials Science and Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466–8555, Japan*

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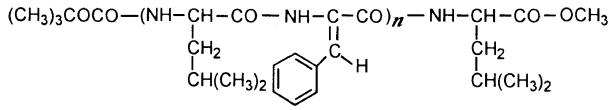
ABSTRACT: In sequential peptides Boc–(L-Leu–ΔPhe)$_n$–L-Leu–OMe **I-*n***, $n=2$—6 (Boc, *t*-butoxycarbonyl; OMe, methoxy), peptides above $n=2$ were already found to form a stable right-handed 3_{10}-helical structure in solution. In the present study, we focused on whether the above chain-length dependence on helical stability retains in the solid state, or not. For this purpose, FT-IR and CD spectra were measured for peptides **I-*n*** in the solid state and in solution. In FT-IR spectra of peptides **I-3** to **I-6** in KBr and in chloroform, two major peaks were observed in amide I absorption band: *i.e.*, first peak at 1665—1655 cm^{-1} assigned to Leu residues in helical segments, and second peak at 1628—1625 cm^{-1} assigned to ΔPhe residues in helical segments. FT-IR pattern of peptide **I-2** differed from those of peptides **I-3** to **I-6** in the solid state and in solution essentially. CD spectra of peptides **I-*n*** in KBr appeared clearly and strongly. Except for peptide **I-2**, peptides **I-3** to **I-6** in the solid state showed exciton couplets with a negative peak at longer wavelengths around 280 nm (assignable to ΔPhe residues), similarly to those in chloroform. The sign of split CD pattern corresponds to a right-handed arrangement of the transition moment: *i.e.*, ΔPhe residues are arranged regularly along a right-handed helical main chain. As a result, a stable helix is formed in the peptides above $n=2$ in the solid state as well as in solution.

KEY WORDS Solid-State Circular Dichroism Measurement / Fourier Transform Infrared Measurement / Sequential Oligopeptides / *Z*-Dehydrophenylalanine / Helical Conformation in Solid State / Chain-Length Effect /

α,β-Dehydroamino acid (unsaturated) residues are naturally present in many peptides having biological activity as well as in some proteins.[1-5] This type of residue shows inherent conformational properties due to its structural features, *e.g.*, the planarity of $C^{\alpha}=C^{\beta}$ double bond and trigonal geometry of α-carbon atom. As for *Z*-dehydrophenylalanine (ΔPhe), the introduction of more than a ΔPhe residue into a peptide chain leads to 3_{10}- and/or α-helical structures.[6-20] Recently, we suggested that polypeptides containing ΔPhe and Pro residues, poly(X–ΔPhe–Pro), take several β-helices as novel types of backbones, from conformational energy calculation and CD measurement.[21] Thus, ΔPhe residue contains useful conformational properties for rational and novel design of peptides.

On the other hand, the conformational properties of ΔPhe residues have not been fully revealed. In our previous study,[19,20] chain-length effects on helical conformations were investigated for sequential peptides **I-*n***:



Boc–(L-Leu–ΔPhe)$_n$–L-Leu–OMe **I-*n***, $n=2$—6
(Boc, *t*-butoxycarbonyl; OMe, methoxy)

As a result, peptides above $n=2$ were found to form a stable right-handed 3_{10}-helical structure in solution.

In the present study, we focused on whether the above chain-length dependence on helical stability retains in the solid state, or not. For this purpose, FT-IR and CD measurement was carried out for peptides **I-*n*** in the solid state. For solid-state CD measurement, Formaggio *et al.*[22] recently reported that screw sense of helical oligopeptides containing chiral C^{α}-methylated residues to induce helical structures was successfully determined by the solid-state CD spectra. In these peptide, *p*-bromobenzamido chromophore was covalently linked to the N-terminal position, and could be coupled with the peptide bond by dipole–dipole interaction, showing the splitting CD pattern around 240 nm. According to exciton chirality method,[23] the helical screw sense could be determined from the sign of exciton couplets. The solid-state CD technique should be suitable for peptides having absorption band above 230—240 nm.[22] Thus, the method will be applied to conformational analysis of peptides **I-*n*** containing ΔPhe residues that show intense absorption maxima around 280 nm.

EXPERIMENTAL

Materials

Peptides **I-*n*** prepared in the previous study[19] were used.

Measurement

CD and UV spectra were recorded simultaneously using a JASCO J-600. According to ref 22, sample for solid-state CD measurement was prepared as follows. Peptide (*ca.* 0.2 mg) and dry KBr (100 mg) were finely ground, and the powder was pressed at 300 kg cm^{-2} for 10 min to prepare a clear and homogeneous disk of a 2 cm diameter. The disk held by a disk holder was placed normal to the light beam. To obtain the average signal of six signal-scan measurements, the disk was rotated

† To whom correspondence should be addressed.

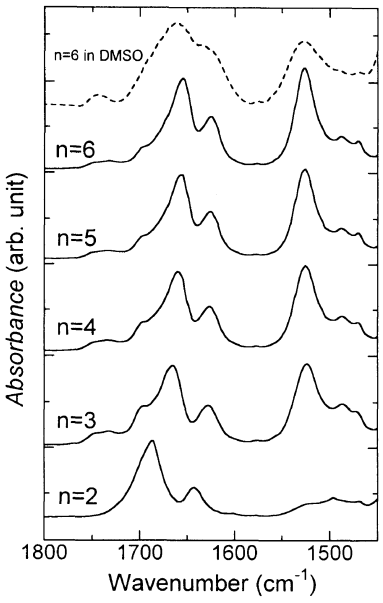


Figure 1. FT-IR spectra (1800—1450 cm^{-1}) of peptides **I-*n*** ($n=2$—6) in chloroform and peptide **I-6** in DMSO.

manually at one every 60°, and then measured (at a rotation angle of 0°, 60°, 120°, 180°, 240°, and 300°). The spectrum of a 100% KBr disk was used as blank. The ordinate of each CD spectrum in KBr was expressed in terms of ellipticity (θ in mdeg), when the corresponding absorption spectrum was normalized to a unity for the maximum absorbance (A_{max}) around 280 nm assignable to ΔPhe residues: *i.e.*, $A_{max}=1.0$. FT-IR spectra were recorded in KBr and in chloroform using a Nicolet FT-IR spectrometer Impact 400 or a JASCO FT/IR-430 spectrometer. In KBr, sample disk was prepared as mentioned above, and a 100% KBr disk was used as blank. In chloroform, peptide solution of 5—10 mM peptide concentration was prepared and transferred to NaCl cell with 0.1 mm cell length, and 100% chloroform was used as blank.

RESULTS AND DISCUSSION

FT-IR Measurement

Figure 1 shows FT-IR spectra of peptides **I-*n*** in chloroform around amide I and II regions, and Table I shows the corresponding peak positions in each spectrum. Little is known about the IR data of ΔPhe-containing peptides, since the properties of C=O stretching band (amide I) for ΔPhe residue is considered to differ from those in saturated amino acid residues due to resonance between C=O and styryl groups in a ΔPhe residue. Previously,[19,20] we revealed that peptides **I-3** to **I-6** took a stable 3_{10}-type helical conformation in solution, based on the ^{1}H NMR and CD spectroscopy, and on conformational energy calculation. Thus, FT-IR spectra of peptides **I-3** to **I-6** in chloroform (Figure 1) can be assigned to amide I and II bands for 3_{10}-type helical structures of sequential peptides containing ΔPhe residues. As the most striking feature of Figure 1 and Table I, two major peaks at amide I band were observed for peptides **I-3** to **I-6** in chloroform: first peak at 1665—1655 cm^{-1}, and second one at 1628—1625 cm^{-1}. The first peak position agrees with that of amide I band

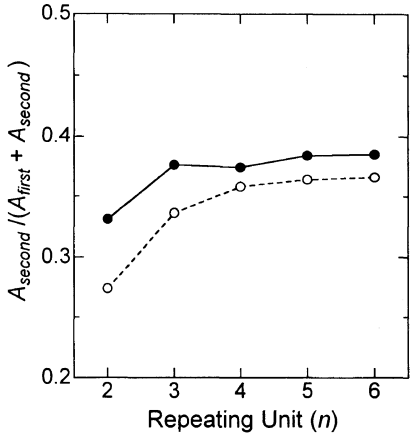
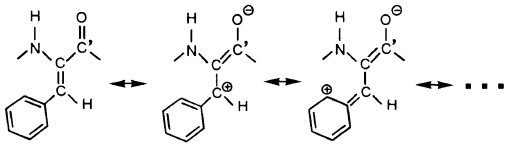


Figure 2. The relation between repeating unit (n) and the contribution of second peak in amide I band in chloroform (○) and in KBr (●): A_{first}, maximum absorbance of first peak; A_{second}, maximum absorbance of second peak in the amide I band.

in a 3_{10}- or α-helix of α-aminoisobutyric acid (Aib)-containing peptides: *e.g.*, 1665 cm^{-1} for 3_{10}-helix of poly(Aib),[24] 1666—1662 cm^{-1} for 3_{10}-helix of Z–(Aib)$_n$–O*t*Bt ($n=8$—10: Z, benzyloxycarbonyl; O*t*Bt, *t*-butoxy) in $CDCl_3$,[25] 1662 cm^{-1} for 3_{10}-helix of and 1659 cm^{-1} for α-helix of Z–(Aib–L-Ala)$_5$–OMe in $CDCl_3$,[25] and 1663 cm^{-1} for 3_{10}-helix of and 1658—1657 cm^{-1} for α-helix of Z–(L-Ala–Aib)$_5$–L-Ala–OMe in $CDCl_3$.[25]

On the other hand, the second peak in the amide I band was not observed for the above Aib-containing peptides. In peptides **I-3** to **I-6**, the intensity of second peak was comparable to that of first peak, and did not tend to decrease with increasing the chain-lengths, as shown in Figure 2. This means that the second peak is not ascribed to irregular parts other than helical segments. Such first and second peaks were also seen in 3_{10}-type helical peptides containing ΔPhe residues: *i.e.*, Boc–(L-Ala–ΔPhe–Aib)$_m$–OMe ($m=2$—4)[26] showed 1655 and 1620 cm^{-1} for peptide $m=4$, 1655 and 1620 cm^{-1} for peptide $m=3$, and 1660 and 1625 cm^{-1} for peptide $m=2$ in the amide I regions. Obviously, the second peak emerged when ΔPhe residues are introduced to helical Aib-containing peptides, which show no second peaks. Therefore, the second peak should be assigned to C=O stretching band of ΔPhe residues incorporated into helical peptides. Compared with saturated amino acid residues, C=O stretching band in a ΔPhe residue is expected to be shifted to lower wavenumbers due to contribution of resonance between C=O and styryl groups within a ΔPhe residue:



The above resonance was experimentally evidenced: The C^{α}–C′ length of ΔPhe residues is 1.50 Å, slightly less than the corresponding distance of 1.53 Å in saturated amino acid residues.[6,27] Also, the value of C′=O bond (1.231 Å) in ΔPhe residues is slightly larger than the value found in Phe residues (1.203 Å).[6,27] In fact, the second peak in the amide I appeared at lower wavenumbers than the first peak, as shown in Figure 1.

Table I. FT-IR data of peptides **I-*n*** in solution and in the sold state

State	*n*	C=O (ester)	C=O (urea)	C=O (amide I) First	C=O (amide I) Second	NH (amide II)
		cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}	cm^{-1}
in $CHCl_3$	6	1732.8	1698.4[a]	1654.7	1625.1	1526.2
	5	1735.0	1698.4[a]	1655.9	1625.8	1525.9
	4	1734.6	1697.7[a]	1660.1	1626.8	1525.3
	3	1732.9 (+1747.5)	1695.9[a]	1665.4	1627.9	1523.9
	2	—	—	1686.4	1642.3	(1496.6)
in DMSO	6	1743.8	—	1661.9	1634.9	1526.9
in KBr	6	1738.7	—	1658.5	1626.4	1525.8
	5	1739.6	—	1658.9	1625.6	1525.7
	4	1740.0	—	1659.4	1626.3	1525.7
	3	1744.3 (+1730.9)	—	1661.1	1626.2	1526.3
	2	Shoulder	—	1684.0	1634.6	(1495.6)

[a] As a shoulder of amide I band.

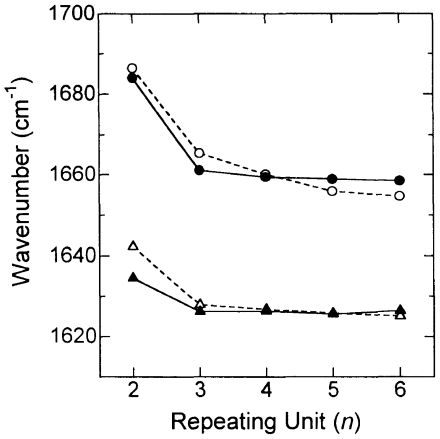


Figure 3. The relation between repeating unit (*n*) and peak position in the amide I band: first (○) and second (△) peaks in chloroform, and first (●) and second (▲) peaks in KBr.

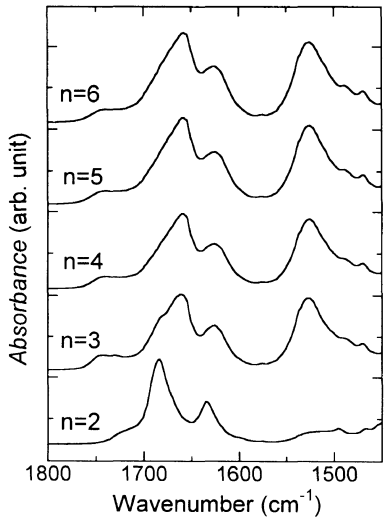


Figure 4. FT-IR spectra (1800—1450 cm^{-1}) of peptides **I-*n*** (*n*=2—6) in KBr.

It should be concluded that the first peak is assigned to Leu residues in helical segments, and the second peak to ΔPhe residues in helical segments. On the other hand, amide II band appeared at 1526—1524 cm^{-1} as single peak. The position was slightly shifted to lower wavenumbers than those of Aib-containing peptides cited above: 1533—1532 cm^{-1} for Z–(Aib)$_n$–O*t*Bt (*n*=8—10), 1536 cm^{-1} for Z–(Aib–L-Ala)$_5$–OMe and Z–(L-Ala–Aib)$_5$–L-Ala–OMe.[25]

As shown in Figure 1, FT-IR pattern of peptide **I-2** differed from those of peptides **I-3** to **I-6** essentially. Figure 3 shows the relation between repeating unit (*n*) and peak position in the amide I band. The first and second peak positions were remarkably shifted to lower wavenumbers in peptides **I-2** to **I-3**, and slightly decreased in peptides **I-3** to **I-6**. Neither first (1686 cm^{-1}) nor second (1642 cm^{-1}) peak positions in peptide **I-2** correspond to those characteristic of amide I band of 3_{10}- and/or α-helical chain (1665—1659 cm^{-1}).[25]

Accordingly, peptide **I-2** contains a conformation other than helix, and a stable helix should be formed in peptides above *n*=2. This conclusion derived from the FT-IR data agreed well with those obtained from ^{1}H NMR and CD spectroscopy, and conformational energy calculation.[19,20] In addition, the first peak position was slightly shifted to lower wavenumbers in peptides **I-3** to **I-6**, suggesting the presence of not only a 3_{10}-helix, but a mixture of 3_{10}-/α-helices.

Also, Figure 1 shows FT-IR spectra of peptides **I-6** in dimethyl sulfoxide (DMSO) around amide I and II regions, and Table I shows the corresponding peak positions in each spectrum. Previously,[20] peptide **I-6** in DMSO was found to show much smaller CD intensity than in $CHCl_3$, which will be shown in Figure 7. This indicates that peptide **I-6** took disordered (random coil) conformations in DMSO. Thus, FT-IR data in DMSO can be assigned to amide bands of peptide **I-6** in random coil conformations. For amide I band, both first and second peaks in DMSO were shifted to higher wavenumbers (7—10 cm^{-1}) than those in $CHCl_3$, while the same peak positions for amide II band were seen in $CHCl_3$ and in DMSO.

Figure 4 shows FT-IR spectra of peptides **I-*n*** in KBr around amide I and II regions, and Table I shows the corresponding peak positions in each spectrum. Essentially, FT-IR data of peptides **I-*n*** in the solid state resem-

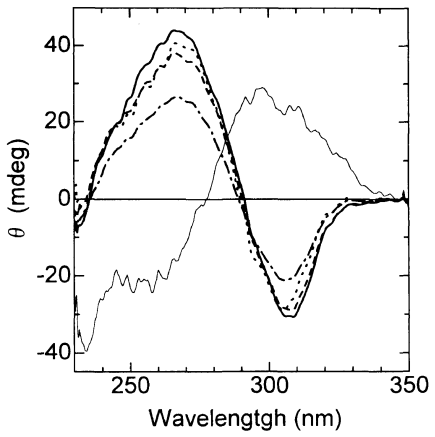


Figure 5. CD spectra of **I-2** (——), **I-3** (—·—), **I-4** (·····), **I-5** (– – –), and **I-6** (▬▬) around 280 nm in KBr.

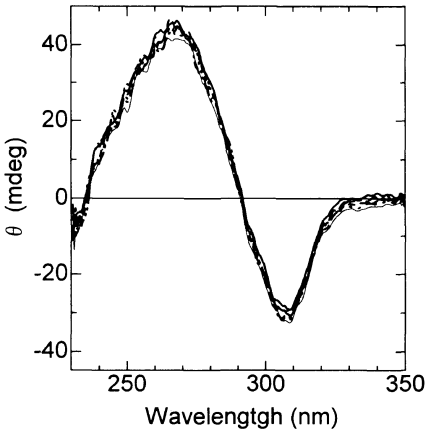


Figure 6. CD spectra of peptide **I-6** in KBr with a rotation angle of 0° (▬▬), 60° (– – –), 120° (·····), 180° (—·—), 240° (—··—), and 300° (——).

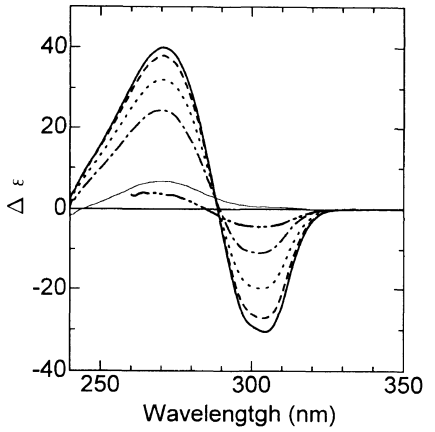


Figure 7. CD spectra of **I-2** (——), **I-3** (—·—), **I-4** (·····), **I-5** (– – –), and **I-6** (▬▬) in chloroform, and **I-6** (—··—) in DMSO around 280 nm.

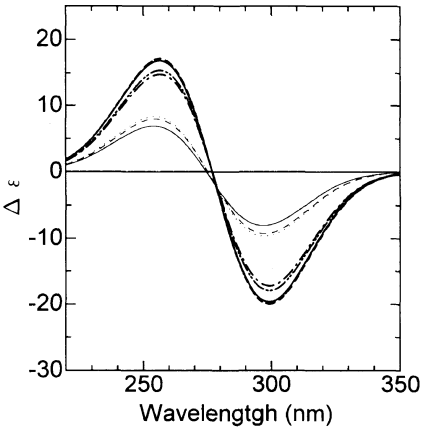


Figure 8. Theoretical CD spectra of peptide **I-4** in the helices (1: $\phi=-44°$, $\psi=-33°$; ▬▬), (2: $\phi=-54°$, $\psi=-28°$; – – –), (3: $\phi=-71°$, $\psi=-18°$; ·····), (4: $\phi=-53°$, $\psi=-36°$; —·—), (5: $\phi=-60°$, $\psi=-30°$; —··—), (6: $\phi=-53°$, $\psi=-52°$; ——), (7: $\phi=-57°$, $\psi=-47°$; – – –), and (8: $\phi=-63°$, $\psi=-42°$; ·····).

bled those in solution. Also, similar chain-length dependence on the peak positions was seen in the solid state and in solution, as shown in Figure 3 and Table I. Thus, it should be concluded that peptide **I-2** does not take a stable helix, but a stable helix is formed in peptides above $n=2$ also in the solid state.

CD Measurement

Peptides containing ΔPhe residues show intense absorption maxima around 220 nm and around 280 nm. The former absorption band precludes a far-UV CD analysis to usually reveal conformations of peptides or proteins. On the other hand, the latter absorption band has been assigned to charge transfer from the highest occupied orbital localized on styryl moiety to the vacant orbital of carbonyl group in a ΔPhe residue.[28,29] The transition moment was estimated from molecular orbital calculation to lie on the styryl-carbonyl line.[26] Thus, most of peptides containing more than a ΔPhe residue will show exciton-type CD couplets, of which the sign and amplitude give useful information for conformational analysis.

Figure 5 shows CD spectra of peptides **I-2** to **I-6** around 280 nm in KBr. All CD spectra appeared clearly and strongly, and CD spectrum of each sample did not change with every 60° rotation essentially, *e.g.*, as shown in Figure 6 for peptide **I-6**. Also, ΔPhe-containing peptides without chiral residue(s) (*e.g.*, Boc–(Aib–ΔPhe)$_4$–Aib–OMe) in KBr gave no signals (only a noisy base line). Accordingly, CD spectra of peptides **I-2** to **I-6** were not caused by artifacts such as light-scattering. Except for peptide **I-2**, peptides **I-3** to **I-6** in the solid state showed exciton couplets with a negative peak at longer wavelengths, similarly to those in chloroform as shown in Figure 7.[19] By applying the exciton chirality method[23] to the present system, the split CD pattern corresponds to a right-handed arrangement of the transition moment: *i.e.*, ΔPhe residues are arranged regularly along a right-handed helical main chain. This is also supported by theoretical CD spectra calculated based on exciton-chirality method.[23] Figure 8 shows theoretical CD spectra of peptide **I-4** in five right-handed 3_{10}-type (4→1 hydrogen bonded) helices (1)—(5) and three right-handed α-type (5→1 hydrogen bonded) helices (6)—(8): (1) $\phi=-44°$, $\psi=-33°$[30]; (2) $\phi=-54°$, $\psi=-28°$[31]; (3) $\phi=-71°$, $\psi=-18°$[32]; (4) $\phi=-53°$, $\psi=-36°$[33]; (5) $\phi=-60°$, $\psi=-30°$[34,35]; (6) $\phi=-53°$, $\psi=-52°$[36]; (7) $\phi=-57°$, $\psi=-47°$[37]; (8) $\phi=-63°$, $\psi=-42°$.[38] In all theoretical spectra, exciton couplets with a negative peak at longer wavelengths were seen for peptides **I-4** in right-handed helices. For the other peptides, similar

theoretical CD patterns were obtained.

On the other hand, peptide **I-2** in the solid state showed exciton-couplets of the opposite sign, while it in chloroform showed highly distorted exciton couplets or positive signals. The change of CD pattern with environments (in the solid state and in solution) means that peptide **I-2** does not take a stable helix, but a stable helix is formed in peptides above $n=2$ in the solid state as well as in solution, which agrees with the results in the preceding section.

CONCLUSIONS

First, FT-IR and CD spectra were measured for sequential oligopeptides containing ΔPhe residues **I-*n*** in the solid state and in solution. As a result, a stable helix is formed in peptides above $n=2$ in the solid state as well as in solution. Namely, the chain-length dependence on helical stability retains in the solid state.

Second, FT-IR data were presented here for ΔPhe-containing oligopeptides in helical conformations. Unlike helical oligopeptides consisting of saturated amino acid residues, two major peaks were observed in amide I band: *i.e.*, first peak at 1665—1655 cm^{-1} assigned to saturated residues in helical segments, and second peak at 1628—1625 cm^{-1} assigned to ΔPhe residues in helical segments.

Finally, solid-state CD measurement could be applied to ΔPhe-containing peptides to reveal their conformations in the solid state. Of course, X-ray crystallographic analysis should be suitable for obtaining their complete conformations. However, the solid-state CD technique will be effective and useful for readily obtaining preliminary conformational data on not only ΔPhe-containing peptides, but also on (poly)peptides containing aromatic chromophores such as arylalanines,[39] particularly on such (poly)peptides difficult to crystallize.

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