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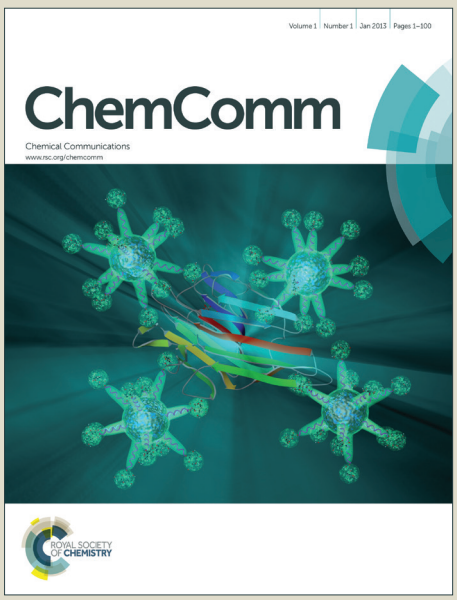

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Pyridyl-Cyclodextrin for Ultra-Hydrosolubilization of [60]Fullerene

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Efficient hydrosolubilizing reagent for [60]Fullerene (C_{60}) was newly developed with γ-cyclodextrin (γ-CD) derivative having triazol-methoxypyridyl moieties at its 6-hydroxyl positions (PCD). Through a solid-state mechanochemical complexation, PCD forms hydrosoluble inclusion complex of C_{60} with a concentration of more than 70 mM. This is approximately 90 times greater than that with non-substituted γ-CD prepared by the same method.

Since the discovery,[1] large number applications of [60]Fullerene (C_{60}) have been reported in the fields of nanotechnology and life science.[2] Originating from their excellent activity, water-soluble C_{60} have been extensively studied for medical applications such as photodynamic therapy, radical scavenger, HIV (human immunodeficiency virus) protease inhibitor, and diagnostic imaging.[2c] However, hydrophobic nature of C_{60} prevents from being well hydrosolubilized at the maximum concentration of 1.3×10^{-11} M only.[3] Therefore, hydrosolubilization of C_{60} by chemical[4] / physical[5] treatments is still an ongoing issue. To address this shortcoming, two strategies have been mainly applied. The most straightforward method is chemical modification by introducing hydrophilic moieties onto C_{60}. For example, polyhydroxylated fullerene (fullerol) improves hydrosolubility up to 37.7 mM.[4c] Unfortunately, such chemical treatments often lead to lose the inherent properties of C_{60}. Alternatively, C_{60} can be hydrosolubilized by forming inclusion complex with hydrophilic host molecules.[6] For instance, γ-cyclodextrin (γ-CD), which is a cyclic oligosaccharide with eight glucose units, uptakes C_{60} into their hydrophobic inner cavity by forming of a 1:2 inclusion complex of C_{60} with two γ-CDs (C_{60}/γ-CD). However, C_{60} does not dissolve in "aqueous" medium whereas γ-CD dissolves well in aqueous medium. To address this incompatible solubility condition, a solid-state mechanochemical process using ball milling have often been employed as a facile but efficient inclusion complexation method. Indeed, C_{60} was hardly uptaken into the γ-CD at a maximum concentration of 0.1 mM,[7] even if solid C_{60} was stirred vigorously in aqueous γ-CD solution. On the contrary, when C_{60} and γ-CD was subjected to mechanochemical complexation in solid state by using ball milling, the resulting C_{60}/γ-CD inclusion complex exhibited relatively higher hydrosolubility up to 1.4 mM.[8] An advantage of the solid-state mechanochemical complexation is that C_{60} can express its inherent physical / chemical properties even though the individual C_{60} is uptaken by the host molecules in aqueous media. However, this method requires excess amounts of γ-CD to stabilize the C_{60}/γ-CD inclusion complex, probably due to the intrinsic lower association ability between C_{60} and γ-CD.[9] Therefore, it remains a big challenge to improve hydrosolubility and stability of the inclusion complex for practical use of C_{60} in biomedical fields.

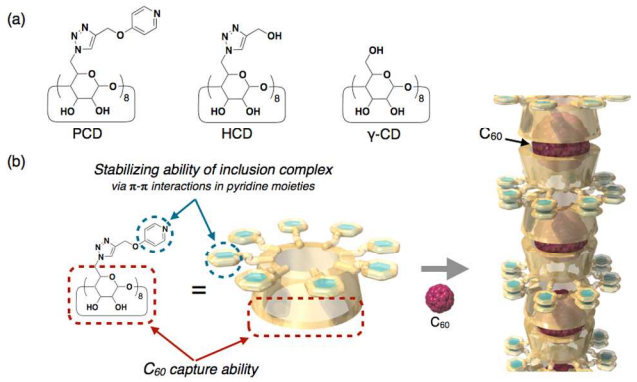


Fig. 1 (a) Chemical structures of cyclodextrin derivative and γ-CD. (b) Schematic illustration of host-guest complexation and hierarchical assembling of inclusion complex of C_{60} with newly synthetic PCD.

In our previous work, we have demonstrated that γ-CD derivative with amino groups at 6-positions favorably formed a 1:2 inclusion complex with the concentration reached to 1.0 mM, in the similar manner of C_{60}/γ-CD.[10a] This suggests that C_{60} is only accessible from the bottom face of 6-amino-γ-CD when it forms the inclusion complex with C_{60}. Thus, chemical modifications at the 6-positions appear not to interfere formation of inclusion complex with C_{60}, as long as a cyclic ring of γ-CD is not vulnerable to structural distortion. Herein, we envisaged that monofacially functionalized γ-CD derivatives at the 6-positions would exhibit higher stability in inclusion complexation with C_{60} and hydrosolubility. Among various candidates,[11] we chose 6-[4-(4-methoxypyridyl)-1*H*-1,2,3-triazol-4-yl]-γ-CD (PCD), the γ-CD derivative with pyridyl groups at its primary face as a prominent γ-CD derivative, which have shown

higher inclusion complexation ability with C_{60} and remarkable hydrosolubility (Fig. 1). Monofacially functionalized γ-CDs were prepared following aforementioned papers.[10] As a facile but efficient preparation of monofacial functionalized γ-CDs, we employed so-called "click chemistry" named Huisgen [3 + 2] cycloaddition. First, primary hydroxyl groups at 6-position of γ-CD were subjected to halogenation followed by conversion to azide group. Subsequently, azide substituted γ-CD was subjected to reaction with two types of alkyne in the presence of Cu (I) ion as a catalyst. The target materials were produced in a quantitative yields of purity > ca. 70 %. As a reference, γ-CD derivative without pyridyl units was prepared with 1-hydroxy-2-propyne (HCD). The obtained γ-CD derivatives were characterized by means of ^{1}H, ^{13}C NMR, 2D NMR (HMQC and HMBC), and MALDI-TOF-MS.

Inclusion complexation of C_{60} with PCD was conducted through the solid-state mechanochemical reaction.[8,12] By using a ball milling, C_{60} was vigorously shaken with equimolar of PCD. The resulting mixture was dissolved in water, and then insoluble portion, mostly un-included C_{60} was removed by centrifugation. UV-vis absorption of C_{60} shows an abrupt onset at 629 nm, along with UV-vis absorption bands at 601, 574, 536, 493, 408, 332, 260, and 214 nm (Fig. S1).[13] Therefore, the combination of transparency in blue and red regions gives homogeneously dissolved C_{60} a distinct purple color to the eye. Under similar experimental conditions, purple color of the C_{60}/PCD solution was much deeper than those of C_{60}/HCD or C_{60}/γ-CD, suggesting a stronger uptake ability of PCD for C_{60} (Fig. 2a). To quantitatively evaluate amounts of C_{60}/PCD in aqueous solution, UV-vis measurements were performed in water at room temperature (Fig. 2b). Here, monitoring an absorption peak of pyridyl group at 264 nm, the molar extinction coefficient of PCD was determined to be 1.31×10^5 cm^{-1} mol^{-1} L (Fig. S2). Furthermore, based on an absorption peak at 332 nm of monomeric C_{60} in 1:2 inclusion complex with γ-CD, the molar extinction coefficient 4.27×10^4 cm^{-1} mol^{-1} L of C_{60} included in aqueous γ-CD solution was adapted from a previous report.[13] Concentration of both C_{60} and PCD in aqueous solution of C_{60}/PCD was evaluated using the fingerprint UV-vis absorption bands for C_{60} and PCD. C_{60} was monitored at 332 nm because of no UV-vis absorption band of PCD in this region. Originating from the pyridyl group, PCD has a typical UV-vis absorption band at 264 nm. Although this PCD band is just beside characteristic absorption band of C_{60} at 260 nm, these two bands were clearly recognizable. To clarify their attributions, subtraction spectra from 264 nm (PCD) to 260 nm (C_{60}) were measured in C_{60}/PCD aqueous solution. Here, absorption band of C_{60} at 332 nm in C_{60}/γ-CD was utilized as an internal standard. Consequently, we selectively extracted the reliable UV-vis absorption signal of PCD from C_{60}/PCD aqueous solution (Fig. S3). Moreover, absence of UV-vis peak at 440 nm suggested that C_{60} did not form aggregation[7] but was well solubilized at even such a high concentration in aqueous medium. Assuming that almost all C_{60} single molecules were uptaken in the PCD cavity to form the C_{60}/PCD inclusion complex, the mole ratio between C_{60} and PCD was estimated to be 1:1.8–2.0. Here, it is immediately noticeable that 72.7 mM of hydrosolubility of C_{60} in the inclusion complex with PCD is much greater than the best solubilizing organic solvent of chrolonaphtalene (70.8 mM).[14] To our knowledge, PCD is the best solubilizing reagent for C_{60} either in aqueous or organic media. In addition, C_{60}/PCD exhibited long stability even under high concentration for at least two months whereas C_{60}/HCD and C_{60}/γ-CD involved precipitation after leaving several minutes to hours.

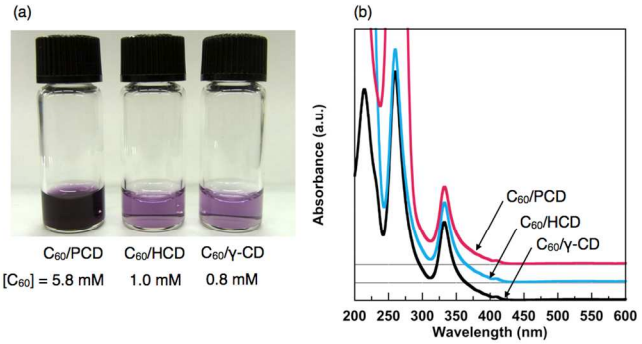


Fig. 2 (a) Photographs of the extracted C_{60} by CD derivatives in water. Concentration of the extracted C_{60} estimated by detecting absorbance at 332 nm of the inclusion complexes. (b) UV-vis absorption spectra of the extracted C_{60}, in which the concentration of C_{60} were fixed at 2.0×10^{-5} M (1 cm cell).

The detailed structure of C_{60}/PCD was further elucidated by NMR analyses. PCD showed characteristic proton signals of H-1, -2, -3, -4, and -5–6 (overlapped peaks) at 4.97, 3.53, 3.86, 3.26, and 3.88–4.02 ppm respectively (Fig. 3a). After inclusion complexation, ^{1}H NMR signals at H-1 and -2 protons shifted to the up-field, but those at H-3, -4, -5, and -6 shifted to the down field (Fig. 3b). Especially, the ^{1}H NMR signals without C_{60} at 7.74 ppm and 6.22 ppm (pyridyl group), and 3.53 ppm (H-2, a hydroxyl group inside the CD cavity) obviously up-field shifted after inclusion complexation to 7.68, 6.15, and 3.45 ppm respectively. Recently, Ikeda and his co-workers succeeded in the high-resolution single crystal structural analysis of 1:2 inclusion complex of γ-CD with mono-*N*-succinoylpyrrolidine functionalized C_{60}, in association with ^{1}H NMR analysis.[15] Significant importance of this work is, utilizing the correlation between crystal structure and ^{1}H NMR chemical shift, accurate feature of the inclusion complex can be predictable from ^{1}H NMR measurements only. Owing to the characteristic ^{1}H NMR chemical shifts at H-2 of PCD and H-1 proton of γ-CD, an abundance ratio of PCD or γ-CD associating with / without inclusion complex with C_{60} was calculated. Consequently, it was evaluated that 94% PCD formed the inclusion complex with C_{60}, whereas only 42% γ-CD formed the inclusion complex, and the remaining 58% γ-CD existed as a non-inclusion form.[16] Compared with C_{60}/γ-CD, it is apparent that the stability of C_{60}/PCD drastically increased by introducing pyridyl groups to γ-CD.

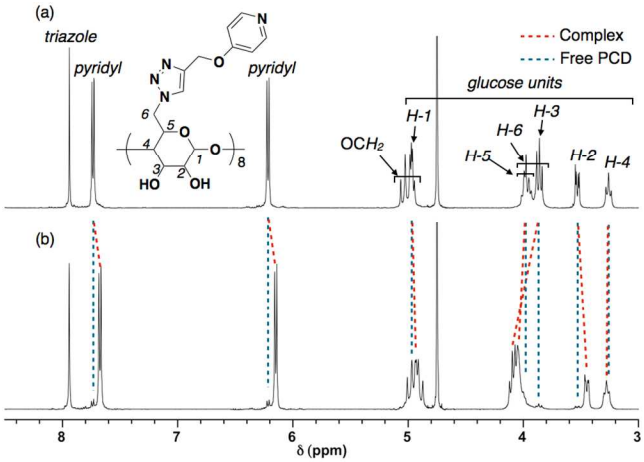


Fig. 3 ^{1}H NMR spectra of (a) PCD and (b) C_{60}/PCD in D_2O (400 MHz, HDO as internal reference).

In addition, after inclusion complexation, pyridyl protons at 7.74 and 6.22 ppm significantly shifted to the up-field by 0.06–0.07 ppm whereas the triazole proton at 7.94 ppm of PCD remained unchanged. Considering that C_{60} is only accessible from the non-functionalized PCD ring, the triazolyl groups appear not to attribute to inclusion complexation with C_{60}. Therefore, changes in chemical shifts of pyridyl groups imply that C_{60}/PCD units associated to the higher-ordered supramoleculer structures, in which the pyridyl groups may act as non-covalent interaction moieties to connect C_{60}/PCD units.

To realize the role of the pyridyl groups of PCD for inclusion complexation with C_{60}, direct observation of C_{60}/PCD was conducted using an atomic force microscopy (AFM)(Fig. 4 and S5). Consequently, it was revealed that C_{60}/PCD inclusion complex spontaneously formed fibril structures. Fig. 4a shows AFM image of drop-cast C_{60}/PCD on a fleshly-cleaved mica surface. The sample was washed with a gentle flow of deionized water. As a result, fibrous structures were observed in the whole observation area (Fig. 4a). However, the fibrous structures drastically transformed to dot-like structures when the sample was vigorously washed with deionized water (Fig. 4b). From the cross-sectional analysis, average height of the dot structures was evaluated to be 2.6 nm (Fig. S5). This value corresponds to 2.4–2.7 nm in the long axis length between pyridyl groups facing two PCDs across C_{60}, which was evaluated from molecular mechanics calculation. The detailed computational study is discussed below. This result indicates that C_{60}/PCD spontaneously associated by non-covalent interaction among pyridyl groups, resulted in formation of fibril structures. Thus, this unusual supramolecular fibrous formation seems to be a key phenomenon to stabilize C_{60}/PCD in aqueous solution, resulted in an extremely high hydrosolubility of C_{60} accommodated in PCD cavities. To further clarify the nature of the

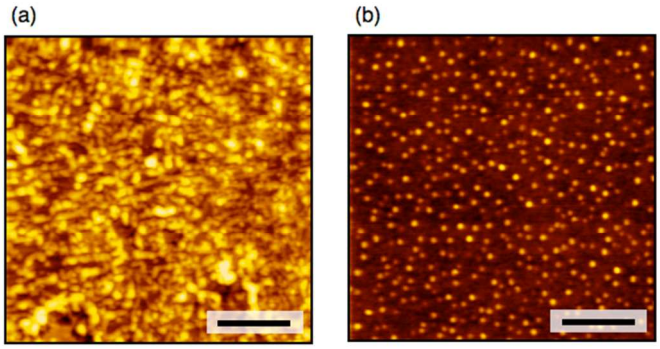
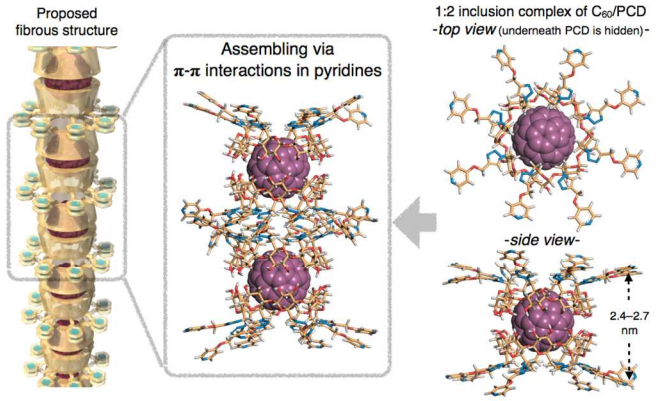


Fig. 4 AFM images of the C_{60}/PCD complex obtained by drop cast of the solution and washed by water on mica. Image (b) was observed at more washed region than (a). The scale bars in the images indicate 500 nm.

supramolecular association behavior of C_{60}/PCD, computational studies with molecular mechanics calculations were performed using Universal force field (Forcite in Materials Studio 4.0, Accelrys Inc., San Diego, CA) (Scheme 1). First, the most stable structures of C_{60}/PCD alone were optimized, in which C_{60} was placed in the pair of PCDs without constraining the position. Here, the triazolyl-pyridyl groups spontaneously spread in a circle. Successively, pyridyl groups were able to interact each other without structural distortion of CD rings. For optimization of fibrous structure, the C_{60}/PCD was placed in the face-to-face manner. To simplify the calculations, two C_{60}/PCD units were employed as a model of fibrous supramolecular architectures. After optimization, C_{60}/PCD was stabilized with the help of inter-unit pyridyl-pyridyl interactions.

As a building block component of supramolecular architecture, aromatic benzene and its derivatives have been regarded as efficient non-covalent binding moieties, due to relatively strong non-covalent π-π interaction. In contrast, recent computational study suggested that pyridyl interaction (−1.691 kcal mol^{-1}) is almost identical to π-π interaction of benzenes (–1.917 kcal mol^{-1}).[17] Thus, by introducing heteroatoms in the benzene ring, stabilization energy of π-π interaction could be widely modulated. To our best knowledge, this simple approach has not been applied for artificial supramoleculer architecture systems whereas the pyridyl-pyridyl interaction plays significant role in biological systems. Thus, it is noteworthy that pyridyl group can be regarded as hydrophilic and non-covalent interaction unit. In addition, it was also beneficial that relatively small structure of pyridyl group can avoid steric repulsion among pyridine rings. Therefore, CD rings were not distorted by functionalization, resulting in stabilization of PCD inclusion complex. Thus, pyridyl group should be regarded as an efficient non-covalent building unit to design the hydrosoluble supramolecular architectures. Here we considered π-π interaction of pyridine group as a dominant interconnecting interaction, but nitrogen atom in pyridyl group appears not to contribute through hydrogen bonding.[18] It is because PCD does not have a proton-donating group adjacent to the pyridyl group.



Scheme 1 Proposed structures of PCD and its multi-step molecular recognitions with C_{60}; the host-guest complexation of PCD to C_{60} followed by the self-assembly of the 1:2 inclusion complex. The models were calculated from Materials Studio (Universal was used as a force field). In them, atom colors orange, white, red and blue represent respectively C, H, O and N.

In conclusion, we prepared pyridyl-terminated γ-CD (PCD) for efficient hydrosolubilizing reagent of C_{60} by using a solid-state mechanochemical complexation with a ball milling. The resulting inclusion complex of PCD and C_{60} exhibited 90 times greater hydrosolubility than the best hydrosolubilizing reagent, non-substituted γ-cyclodextrin. C_{60}/PCD stabilized by forming fibrous architecture, in which pyridyl groups acted as non-covalent binding moieties through relatively strong pyridyl-pyridyl interaction. The present hierarchical supramolecular approach for hydrosolubilization of C_{60} may respond not only for immediate needs in medical application such as drug delivery system, but also provide novel methodology to design the sophisticated supramolecular architectures.

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† Electronic Supplementary Information (ESI) available: Synthesis detail of cyclodextrin derivatives, UV-vis, NMR, AFM data for PCD and C_{60}/PCD. See DOI: 10.1039/c000000x/

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