Supporting Information

Cucurbit[6]uril-based Polymer Nanocapsules as a Non-covalent and Modular Bioimaging Platform For Multimodal *In vivo* Imaging

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General Procedures

All the reagents and solvents employed were commercially available and used as supplied without further purification. Cyclic RGDyk peptide was obtained from PEPTRON (Daejeon, Korea). Dialysis was performed using Thermo snakeskin pleated dialysis tubings (MWCO: 7,000 and 10,000) and Spectra/Por Biotech Regenerated Cellulose (MWCO: 2,000). The nuclear magnetic resonance (NMR) spectra were acquired at 298 K on a Bruker Advance 850 MHz. Mass (MS) analysis was performed using a LTQ-XL mass spectrometer (Thermo Fisher Scientific, Inc.) equipped with an electrospray ionization (ESI) source. For automatic measurement and data analysis, Xcalibur software was used (Thermo Fisher Scientific, Inc.). MALDI-TOF spectra were acquired on an Autoflex speed (Bruker). UV-Vis absorption spectra were collected by an Agilent Cary 5000 UV-Vis-NIR Spectrophotometer. Fluorescence emission spectra were collected by RF-5301PC Spectrofluorophotometer (Shimadzu). The surface charges and sizes of the nanoparticles were determined using a Zetasizer Nano ZS (Malvern Instruments Ltd.) at 25 °C. High-resolution TEM images were recorded on FEI-Titan G2 60-300 electron microscope at 80 kV. Copper-64 was produced by KIRAMS (Seoul, Korea) via the $^{64}Ni(p,n)^{64}Cu$ nuclear reaction using a MC 50 cyclotron (Scanditronix, Sweden). *In vivo* positron emission tomography (PET) imaging was performed using an Inveon Trimodality image system (Siemens) at the Pohang Center of Evaluation of Biomaterials (Pohang Technopark). *Ex vivo* near-infrared (NIR) imaging was performed using an IVIS spectrum small animal *in vivo* imaging system (Caliper Life Science) located at the Pohang Center of Evaluation of Biomaterials (Pohang Technopark).

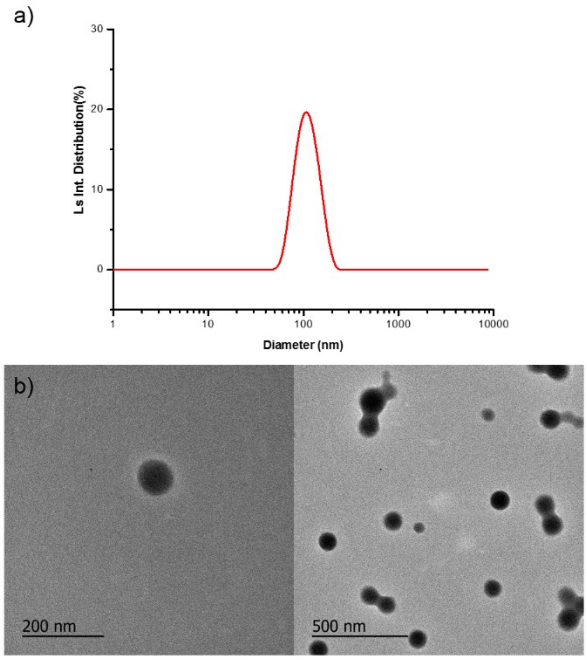


Fig. S1 Characterization of CB[6]PNs. a) size distribution of CB[6]PNs[S1] determined by DLS (110 ± 30 nm) in DI water and b) TEM images.

In vitro stability of CB[6]PNs in a physiological condition

A solution of CB[6]PNs in DI water (0.5 mM, 100 μL) was added to a solution of 10% FBS (800 μL) in DI water. The diameter of the sample was measured by DLS at different incubation time (0 h and 12 h).

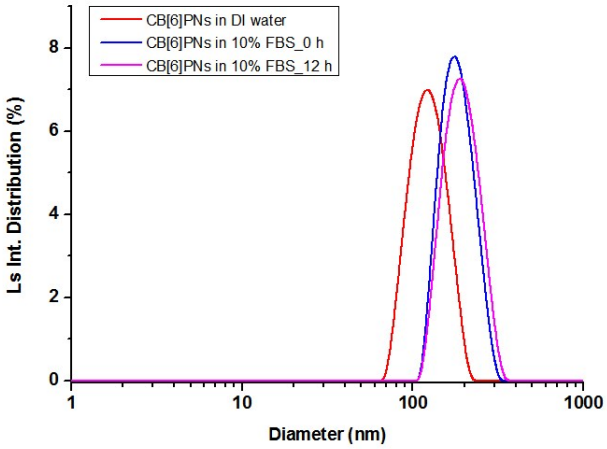


Fig. S2 DLS results of CB[6]PNs in DI water and a serum condition at different incubation time.

Cell culture

CT26 (mouse colon cancer cells) was cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone). Each media contains 10% fetal bovine serum (FBS, Hyclone) and 1%

penicillin and streptomycin. Each cell was incubated at 37 °C in a 5% CO_2 humidified incubator.

Cell viability test (MTT assay)

The cytotoxicity and cell proliferation effects of CB[6]PNs were examined by using a MTT assay. CT26 cells were seeded onto a 96-well plate at a density of 1×10^4 cells per well using DMEM media (200 μL) containing 10% FBS and 1% penicillin and streptomycin. The plate was incubated at 37 °C under a humidified atmosphere with 5% CO_2. After 24 h, a solution of CB[6]PNs (20 μL) with different concentrations (from 0 to 170 μg mL^{-1}) was treated to the wells, and was incubated for 4 h. The cells were washed with PBS (200 μL), and fresh DMEM media (200 μL) was added to the each well. After an additional 20 h incubation, fresh media (200 μL) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg mL^{-1}) were added to the wells. After 4 h of incubation, the media was removed and DMSO (200 μL) was added to the wells to dissolve the purple formazan crystals. The absorption at 570 nm was measured using a microplate spectro-fluorometer (VICTOR^3 V Multilabel Counter; PerkinElmer, Well-esley, MA, USA). The cytotoxicity of CB[6]PNs was measured to be 84 ± 3% (n = 5).

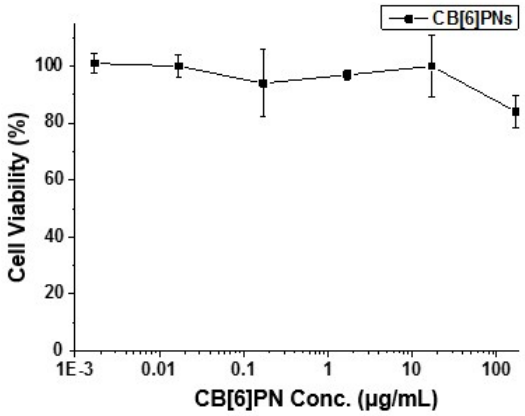


Fig. S3 Cytotoxicity test of CB[6]PNs at different concentrations

In vivo models

All the animal experiments were approved by the POSTECH Biotech Center Ethics Committee. CT26 cells were inoculated subcutaneously (s.c.) at a density of 1×10^5 cells per mouse into the flank of each female Balb/c mice weighing 20 g. The cancer cells were inoculated for approximately 10 days until the tumors reached 5-10 mm in size.

Preparation of stock solutions of spmd-tagged functionalities

Stock solution of FITC-spmd (**1**)[S2] (1.9 mM)**:** FITC-spmd (**1**) (1.0 mg, 1.9 μmol) in DI water (1.0 mL)
Stock solution of Cy7-spmd (**2**) (1.5 mM)**: 2** (1.0 mg, 0.7 μmol) in DI water (1.0 mL)
Stock solution of NOTA-spmd (**3**) (22.0 mM)**: 3** (13.0 mg, 22.0 μmol) in DI water (1.0 mL)
Stock solution of cRGDyK-PEG-spmd (**4**) (0.5 mM)**: 4** (1.5 mg, 0.5 μmol) in DI water (1.0 mL)

Stock solution of PEG-spmd (**5**) (14.3 mM): **5** (30.8 mg, 14.3 μmol) DI water (1.0 mL)
Stock solution of spermidine (**spm**) (60.0 mM): spermine tetrahydrochloride (21.0 mg, 60 μmol) in DI water (1.0 mL)

Accessibility of CB[6] portals on CB[6]PNs

A solution of FITC-spmd (**1**) (2.7 μL from the stock solution of **1**) and Cy7-spmd (**2**) (33.8 μL from the stock solution of **2**) were added to a solution of CB[6]PNs (0.5 mM, 200 μL) in DI water. After incubation for 2 h, the mixture was dialyzed (MWCO: 10,000) against water for 3 h to provide (**1**+**2**)@CB[6]PNs. The successful anchoring of **1** and **2** on CB[6]PNs was confirmed by observing the characteristic fluorescence emission at 520 nm and 770 nm for FITC (ex. 490 nm) and Cy7 (ex. 700 nm), respectively.

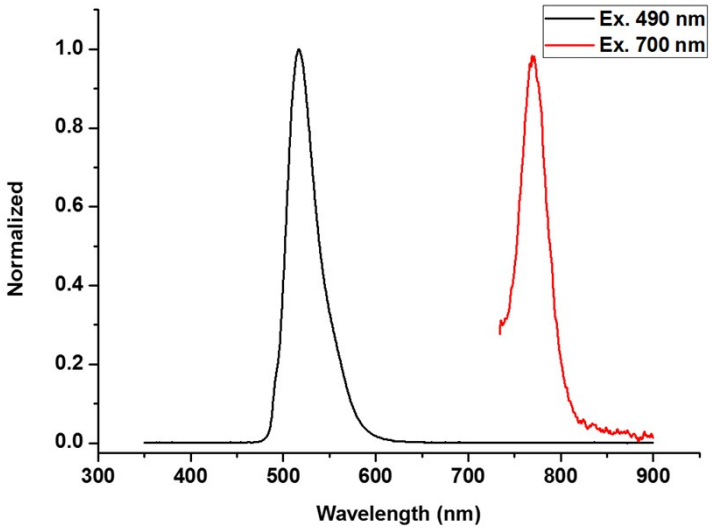


Fig. S4 Fluorescence spectra of (**1**+**2**)@CB[6]PNs.

Control of the non-covalent surface modification

A stock solution of **1** (2.7 μL, 5.4 μL, 10.7 μl and 21.5 μl) was added to a solution of CB[6]PNs (2 mL, 25 μM) in DI water to provide the samples of CB[6]PNs containing 10, 20, 30, 40 and 80 mol% of **1** (to CB[6] on CB[6]PNs) in water. After incubation of the samples with different concentration of **1** at r. t. for 2 h, the resulting samples were dialyzed (MWCO 7000) against water for 6 h. Then, the fluorescence of the released **1** from CB[6]PNs was measured by fluorimeter (excitation: 490 nm, emission: 515 nm). The concentrations of **1** onto the CB[6]PNs, was calculated by subtraction of the released concentration of **1** from that of the initial one (Table S1).

Initially treated FITC-spmd (mol% to CB[6] in CB[6]PNs)	Anchored FITC-spmd (mol% to CB[6] in CB[6]PNs)	Percentage of anchoring
10	9.1	91%
20	19.1	95%
40	37.7	94%
80	75.7	95%

Table S1 Percentage of the anchoring FITC-spmd (**1**) on CB[6]PNs.

Anchoring stability of tag-spmd on CB[6]PNs

In serum conditions: A solution of **1** (3.2 μL from the stock solution of **1**) was added to DI water (2.6 mL) in a vial to provide a diluted solution of **1** (2.3 μM). A solution of **1** (3.2 μL from the stock solution of **1**) was added to a solution of CB[6]PNs (0.1 mM, 2.6 mL) in DI water and dialyzed (MWCO: 7,000) against DI water to provide **1**@CB[6]PNs. A solution of **1**@CB[6]PNs (2.0 mL) or the diluted solution of **1** (2.3 μM, 2.0 mL) was transferred into 50% FBS solution (100 mL) in PBS buffer in a beaker with a cap and was kept at 4 °C. The fluorescence intensity of the dialyzed solution of **1**@CB[6]PNs or **1** was measured at different time intervals (ex. = 470 nm, em. = 515 nm). After 44 h, excess amount of spermine (230 μM) to CB[6]PNs was added into the solution of **1**@CB[6]PNs.

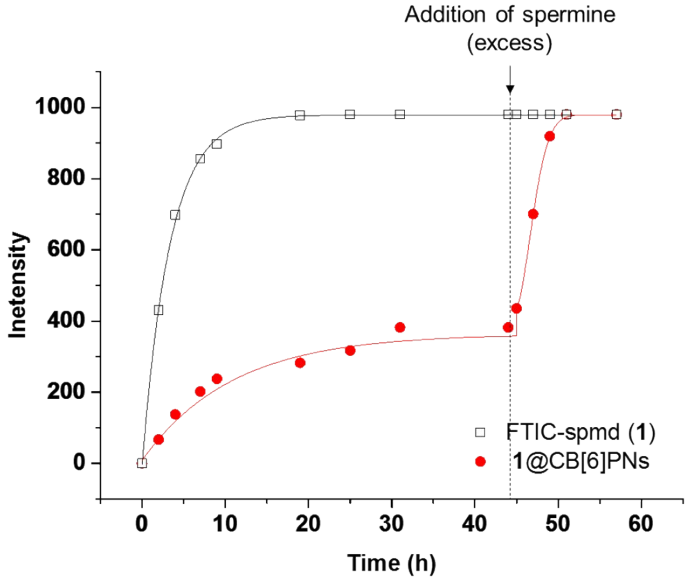


Fig. S5 *In vitro* release profiles of **1** non-covalently anchored to CB[6]PNs (red closed circle) and free molecule, **1** (black open square) in a serum condition.

In *in vivo* conditions: A solution of **1** (41.7 μL from the stock solution of **1**) was added in DI water to provide a diluted solution of **1** (0.3 mM, 250 μL). A solution of Cy5-CB[6]PNs (0.5 mM, 208 μL, see the below for the preparation) was added in DI water to provide a diluted solution of Cy5-CB[6]PNs (0.4 mM, 250 μL) in water. A solution of **1** (41.7 μL from the stock solution **1**) was added into a solution of Cy5-CB[6]PNs (0.5 mM, 208 μL) to provide **1**@Cy5-CB[6]PNs (0.4 mM, 250 μL) in DI water. The resulting solutions of **1**, Cy5-CB[6]PNs and **1**@Cy5-CB[6]PNs were intravenously injected to Balb/c mice. After 30 m and 1 h, the mice

were euthanized via CO_2 asphyxiation. The organs were dissected out and visualized by IVIS imaging system (ex. = 500 nm, em. = 540 nm for **1** and ex. = 640 nm, em. = 700 nm for Cy5).

^{64}Cu labelling

$^{64}CuCl_2$ (6 mCi) in HCl was added to a solution of **3** (300 μL of the stock solution of **3**) under pH 7.5 and the mixture was incubated at r.t. for 30 min. After purification by a PD10 column cartridge, the radioactivity of the resulting solution was measured by a dose calibrator (yield: 62%).

In vivo PET/NIR multimodal imaging experiments

The mice were randomly divided into 3 groups and intravenously injected with the materials. After 12 h of the injection, the mice were first scanned using PET by a preclinical microPET/CT (Siemens Inveon), and subsequently sacrificed. The fluorescence emission (ex. = 710 nm, em. = 780 nm) of Cy7 from the whole body and each organ was obtained and analyzed with IVIS spectrum small-animal *in vivo* imaging system (Caliper Life Sciences).

Synthesis

Cy5-CB[6]PNs: TCEP (7.2 mg, 25 μmol) and *N*-(2-aminoethyl)maleimide hydrochloride (22.1 mg, 125 μmol) were added to a solution of CB[6]PNs[S2] (5.0 mL, 2.5 μmol) in water. After incubation for 3 h, amine functionalized CB[6]PNs were purified by dialysis (MWCO: 10, 000) against water for 3 h. Sulfo-cyanine 5 NHS ester (0.2 mg, 0.3 μmol) was added to the solution of amine functionalized CB[6]PNs (5.0 mL, 2.5 μmol). After incubation for 3 h, the product was purified by dialysis (MWCO: 10,000) against water for 3 h. The average diameter of Cy5-CB[6]PNs were measured by DLS (150 ± 70 nm) (Fig. S6a). The successful conjugation of Cy5 to CB[6]PNs was confirmed by observing the characteristic fluorescence emission of Cy5-CB[6]PNs at 650 nm (ex. 575 nm) (Fig. S6b).

1@Cy5-PNs: A solution of Cy5-CB[6]PNs (0.5 mM, 208.3 μL) was added to a solution of FITC-spmd (**1**) (41.7 μL from the stock solution of **1**). After incubation for 3 h, the product was purified by dialysis (MWCO: 10,000) against water for 3 h. The average diameter of **1**@Cy5-CB[6]PNs were measured by DLS (120 ± 40 nm) (Fig. S6a). The characteristic fluorescence emission of **1** at 525 nm (ex. 450 nm) and an fluorescence emission at 650 nm for Cy5-CB[6]PNs (ex. 575 nm) was observed, confirming successful anchoring of **1** onto Cy5-CB[6]PNs (Fig. S6b).

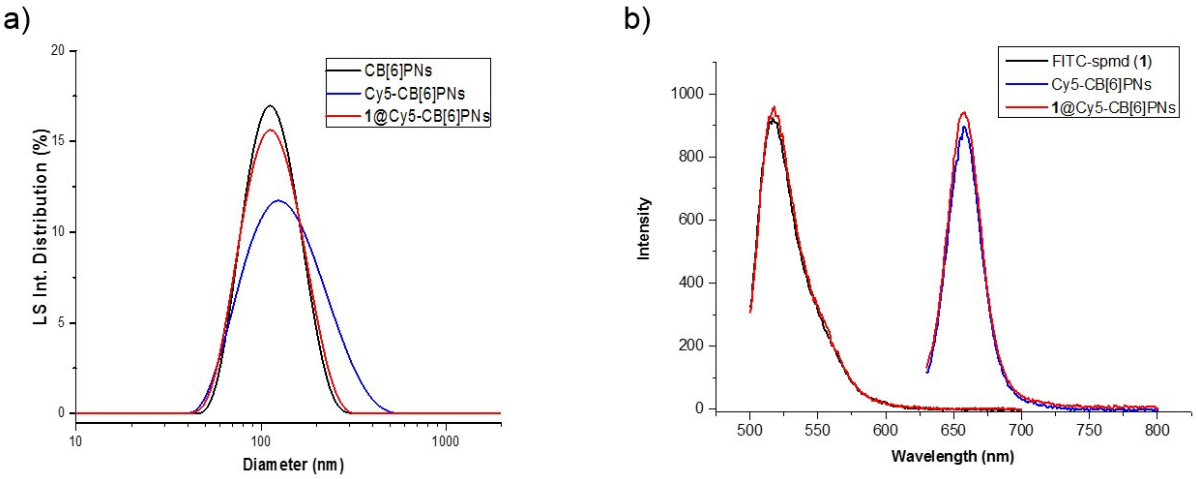


Fig. S6 Characterization of **1**@Cy5-CB[6]PNs by a) DLS, b) zeta potential measurements, and d) UV/Vis absorption spectroscopy.

Biodistribution: **1**@Cy5-CB[6]PNs (1.4 mg mL^{-1}, 250 µL) was intravenously injected into a mouse. The organs (liver, kidney, lung, heat and spleen) were extracted from the mouse after 1 h of the injection. FITC fluorescence emission from the organs measured by IVIS (ex. = 500 nm, em. = 540 nm, Fig. S7).

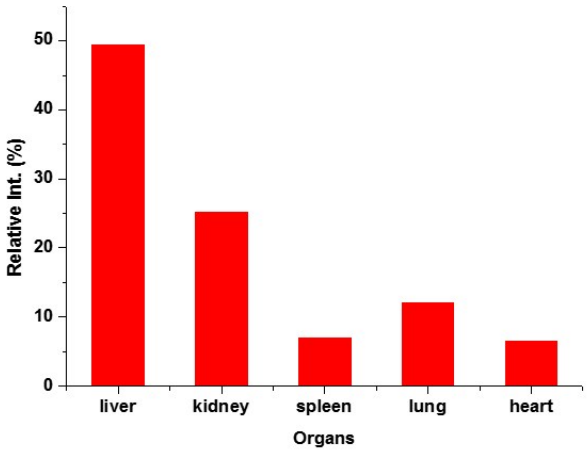


Fig. S7 Biodistribution of **1**@Cy5-CB[6]PNs based on relative fluorescence intensity of the extracted organs.

Histology

A solution of CB[6]PNs in DI water (0.5 mM, 100 µL) was intravenously injected to a Balb/c mouse. After 12 h of injection, the mouse was euthanized via CO_2 asphyxiation and organs were dissected out. After fixation of the organs using formalin for 24 h, the organs were washed thoroughly with water and ethanol, and embedded in paraffin wax for preservation. The formalin-fixed paraffin embedded organs were sectioned at 4 µm thickness using a Finesse ME microtome (Thermo Fisher Scientific). The sliced sections of the fixed organs were stained

with hematoxylin and eosin (H&E) and recorded images of entire sections by an optical microscopy (LEICA DMI6000 B) with 40 × magnification.

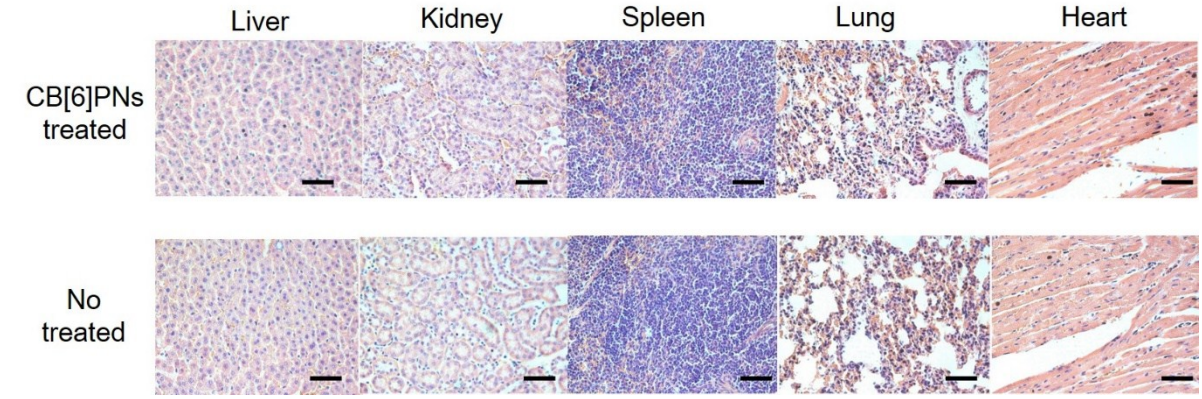


Fig. S8 Hematoxylin and eosin (H&E)-stained sections of major organ tissues extracted from of the mouse treated with or without CB[6]PNs (scale bar = 20 μm)

In vitro **cell imaging experiments**

A solution of **1** (0.4 μL) was added to DI water (200 μL). **1** (0.4 μL from the stock solution of **1**) was added to a solution of CB[6]PNs in DI water (25.0 μM, 200 μL) to obtain **1**@CB[6]PNs. Solutions of **1** (0.4 μL from the stock solution of **1**) and **4** (1.0 μL from the stock solution of **4**) were added to CB[6]PNs in DI water (25.0 μM, 200 μL) to obtain (**1**+**4**)@CB[6]PNs. CT26 cells (20,000 cells per well) were seeded onto glass coverslips placed in a 12-well culture plate and incubated overnight. Medium was replaced by fresh serum-free medium (450 μL) containing each sample (50 μL). After 12 h of incubation, the cells were washed with PBS and fixed with 10% neutrally buffered formalin (NBF) for overnight in 4 °C refrigerator. The fixed cells on the coverslip were mounted by Vectashield antifade mounting medium and intracellular uptake of each sample was visualized by fluorescence microscopy with excitation at 495 nm for **1**.

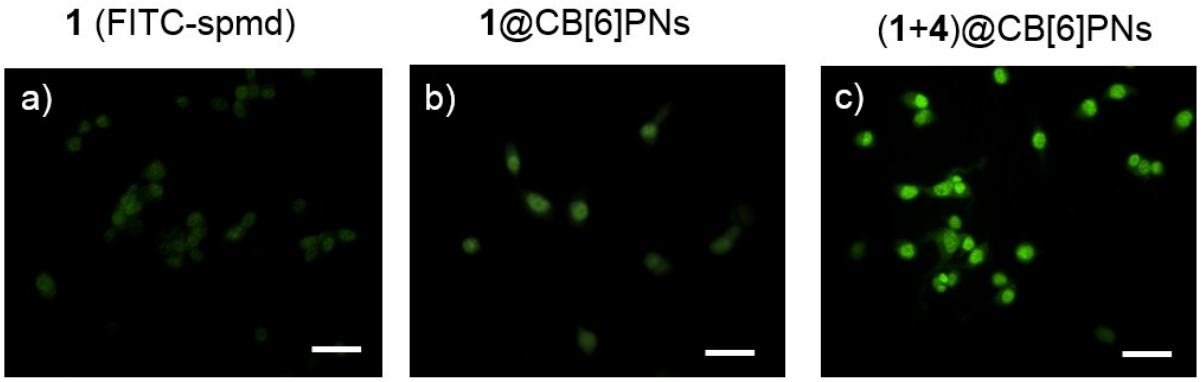
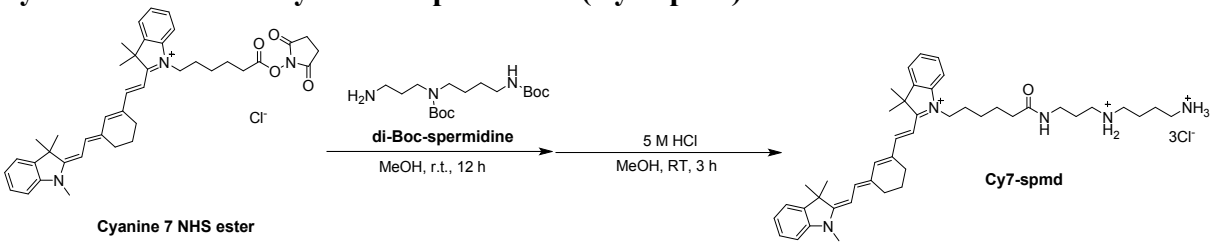
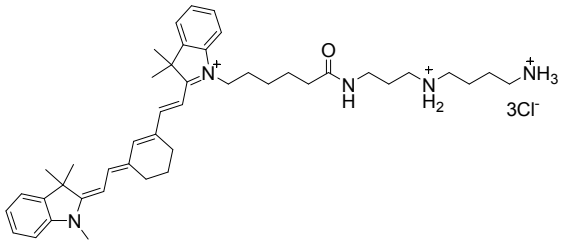


Fig. S9 Fluorescence microscopy images of CT26 cells treated with **1**, **1**@CB[6]PNs and (**1**+**4**)@CB[6]PNs. (scale bar = 20 μm). These images showed enhanced intracellular uptake of (**1**+**4**)@CB[6]PNs into CT26 cells compared to that of **1** or **1**@CB[6]PNs, which supports that **4** allows CB[6]PNs to have cancer targeting ability. In addition, these results suggested that CB[6]PNs can have desired multifunctionalities on-demand upon decoration of the spermidine-conjugated functionalities such as imaging probe (**1**) and targeting moiety (**4**) on CB[6]PNs.

Synthetic scheme of cyanine 7-spermidine (Cy7-spmd):

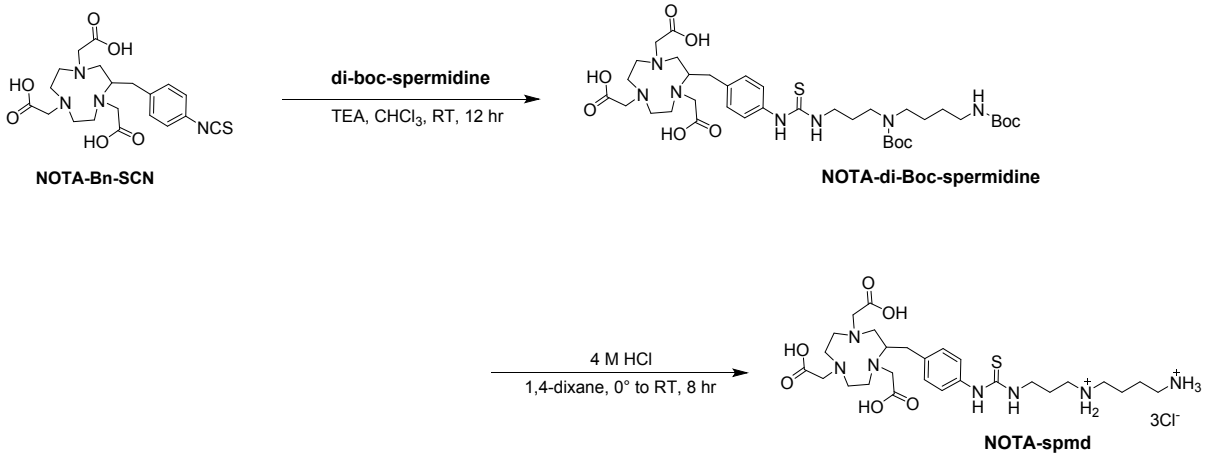


1-(6-((3-((4-aminobutyl)amino)propyl)amino)-6-oxohexyl)-3,3-dimethyl-2-((E)-2-((E)-3-(2-((E)-1,3,3-trimethylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-3H-indol-1-ium (Cy7-spmd)

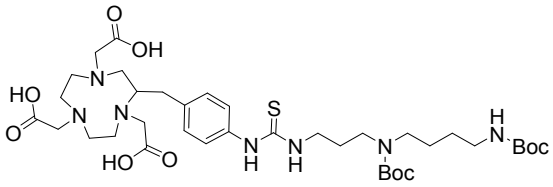


Cyanine 7 NHS ester (5.0 mg, 7.7 μmol) was added to a solution of di-Boc-spermidine[S3] (2.9 mg, 8.5 μmol) in MeOH (4.0 mL) and stirred at room temperature for 12 h. To the reaction mixture, HCl (1.0 mL, 5 M, 5.0 mmol) was added, and was stirred at room temperature for 3 h. The product was purified by recrystallizing the reaction mixture from diethyl ether three times to afford Cy7-spmd (4.4 mg, 84%). HRMS-ESI (m/z): $[M]^+$ calcd for $C_{44}H_{62}N_5O$, 676.4954; found, 676.4954.

Synthetic scheme of NOTA-spermidine (NOTA-spmd):

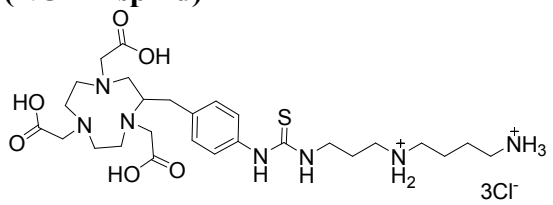


2,2',2''-(2-(4-(3-(3-((tert-butoxycarbonyl)(4-((tert-butoxycarbonyl)amino)butyl)amino)propyl)thioureido)benzyl)-1,4,7-triazonane-1,4,7-triyl)triacetic acid (NOTA-di-Boc-spermidine)



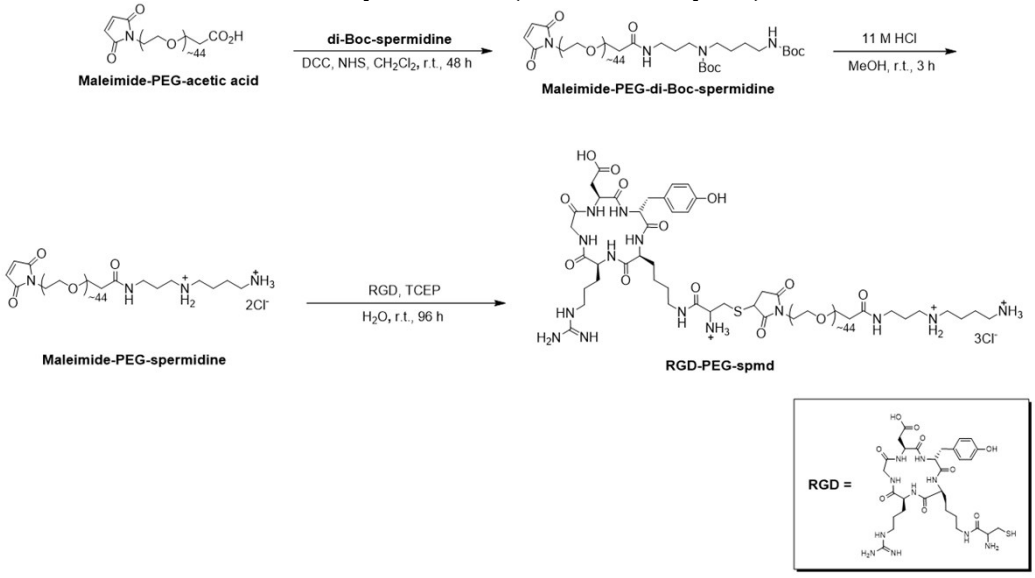
A mixture of 2-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA-Bn-SCN) (0.10 g, 0.18 mmol) and di-Boc-spermidine (0.06 g, 0.18 mmol) in $CHCl_3$ (5.0 mL) containing triethylamine (TEA; 54 mg, 0.54 mmol) was stirred overnight at room temperature. The product was purified by recrystallizing the reaction mixture two times from diethyl ether to afford NOTA-di-Boc-spermidine (150 mg, 85%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.35-7.29 (m, 4H), 3.97-3.90 (m, 4H), 3.62-3.58 (m, 2H), 3.52-2.72 (m, 27H), 1.83-1.82 (m, 2H), 1.81-1.58 (m, 4H), 1.47 (s, 18H), 1.45 (s, 18H); MS-ESI (m/z): $[M + H]^+$ calcd for $C_{37}H_{62}N_7O_{10}S$, 796.42; found, 796.42.

2,2',2''-(2-(4-(3-(3-((4-aminobutyl)amino)propyl)thioureido)benzyl)-1,4,7-triazonane-1,4,7-triyl)triacetic acid (NOTA-spmd)

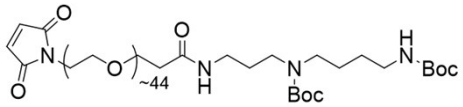


A solution of HCl (1.0 mL (4 M), 4.0 mmol) was added to NOTA-di-Boc-spermidine (150 mg, 0.19 mmol) in 1,4-dioxane (5.0 mL) and the reaction mixture was kept under stirring at room temperature for 8 h. After completion of the reaction, the solvent was removed *in vacuo* and the product was purified by preparative HPLC [Solvents: 1% TFA water (A) and acetonitrile (B); gradient: 0 to 80% of B for 60 min]. The target product was eluted at R_f = 35 min (103 mg, 92%). HRMS-ESI (m/z): calcd for $[M + H]^+$ $C_{27}H_{46}N_7O_6S$, 596.3228; found, 596.3230.

Synthetic scheme of RGD-PEG-spermidine (RGD-PEG-spmd):

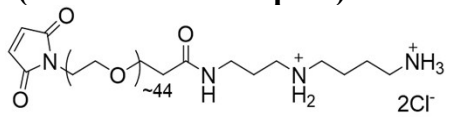


Maleimide-PEG-di-Boc-spermidine (Maleimide-PEG-di-Boc-spmd)



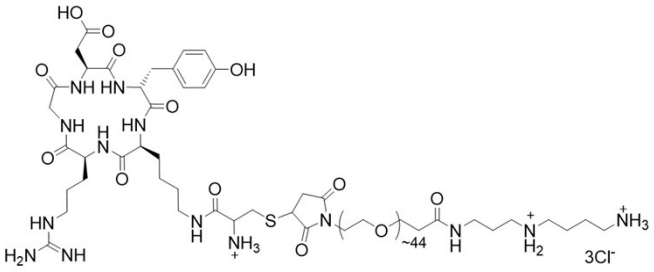
Maleimide-PEG_{2k}-COOH (20.0 mg, 11 μmol), dicyclohexylcarbodiimide (DCC; 6.0 mg, 29.1 μmol) and *N*-Hydroxysuccinimide (NHS; 3.0 mg, 29.1 μmol) and di-Boc-spermidine (4.5 mg, 13.2 μmol) was added in DCM (5.0 mL) and the solution was stirred for 2 d at room temperature. After removing the precipitate by filtering, the filtrate was dried *in vacuo* and the product was purified by dialysis against DI water (MWCO: 2,000) for 48 h to afford Maleimide-PEG-di-Boc-spmd (21.03 mg, 90%) as characterized by MALDI-TOF mass analysis (see below Fig. S10).

Maleimide-PEG-spermidine (Maleimide-PEG-spmd)



The *N*-Boc deprotection of maleimide-PEG-di-Boc-spermidine was carried out at 160 °C for 4 h. The product, maleimide-PEG-spmd was purified (15.1 mg, 78%) by dialysis against DI water for 48 h using a cellulose membrane (MWCO: 2,000) and characterized by MALDI-TOF mass analysis (See below Fig. S10).

RGD-PEG-spermidine (RGD-PEG-spmd)



Maleimide-PEG_{2k}-spmd (6.0 mg, 3.2 μmol), thiolated cRGDyK (2.5 mg, 3.5 μmol) and tris(2-carboxyethyl)phosphine (TCEP; 0.9 mg, 0.35 μmol) were added in DI water (5 mL) and stirred for 4 d. The final product was purified (5.8 mg, 73%) by dialysis (MWCO: 2,000) against water for 48 h and characterized by MALDI-TOF mass analysis (See below Fig. S10).

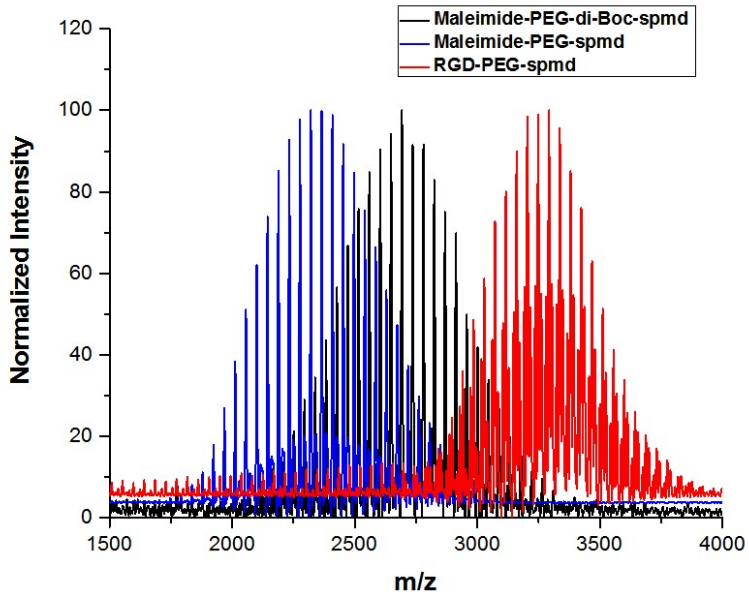
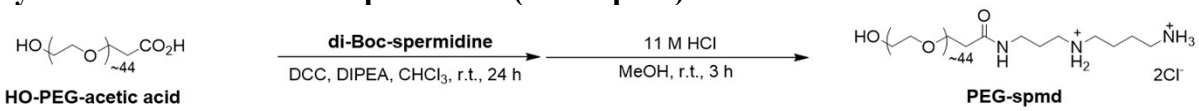
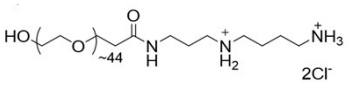


Fig. S10 MALDI-TOF mass spectra of Maleimide-PEG-di-Boc-spmd (black), Maleimide-PEG-spmd (blue) and RGD-PEG-spmd (red).

Synthetic scheme of PEG-spermidine (PEG-spmd):



PEG-spermidine (PEG-spmd)



A mixture of HO-PEG_{2k}-acetic acid (100 mg, 50 µmol) and DCC (21 mg, 100 µmol) in CH_2Cl_2 (4 mL) containing DIPEA (9.6 mg, 75 µmol) was stirred overnight at room temperature. To the reaction mixture, di-Boc-spermidine (25.0 mg, 75 µmol) was added and stirred for 24 h at room temperature. The reaction mixture was first concentrated to remove CH_2Cl_2. Then, MeOH (2.0 mL) and HCl solution (2 mL (11 M)) were added to the concentrate and the reaction mixture was stirred for 3 h at room temperature. After completion of the reaction, the solvent was removed *in vacuo* and the product was purified (23 mg, 44%) by dialysis (MWCO: 2,000) against water for 48 h and characterized by MALDI-TOF mass analysis (See below Fig. S11).

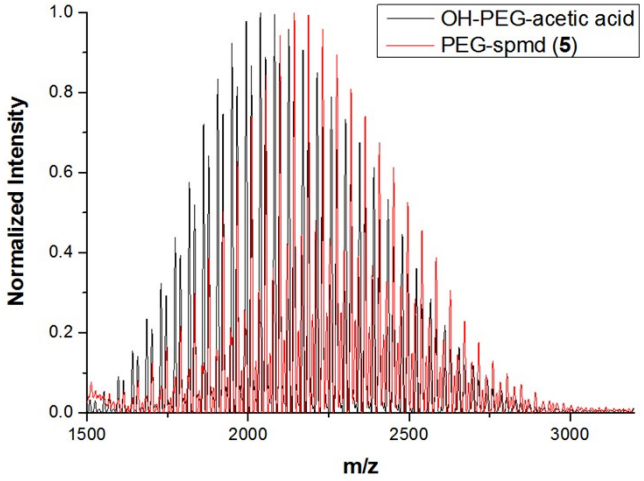
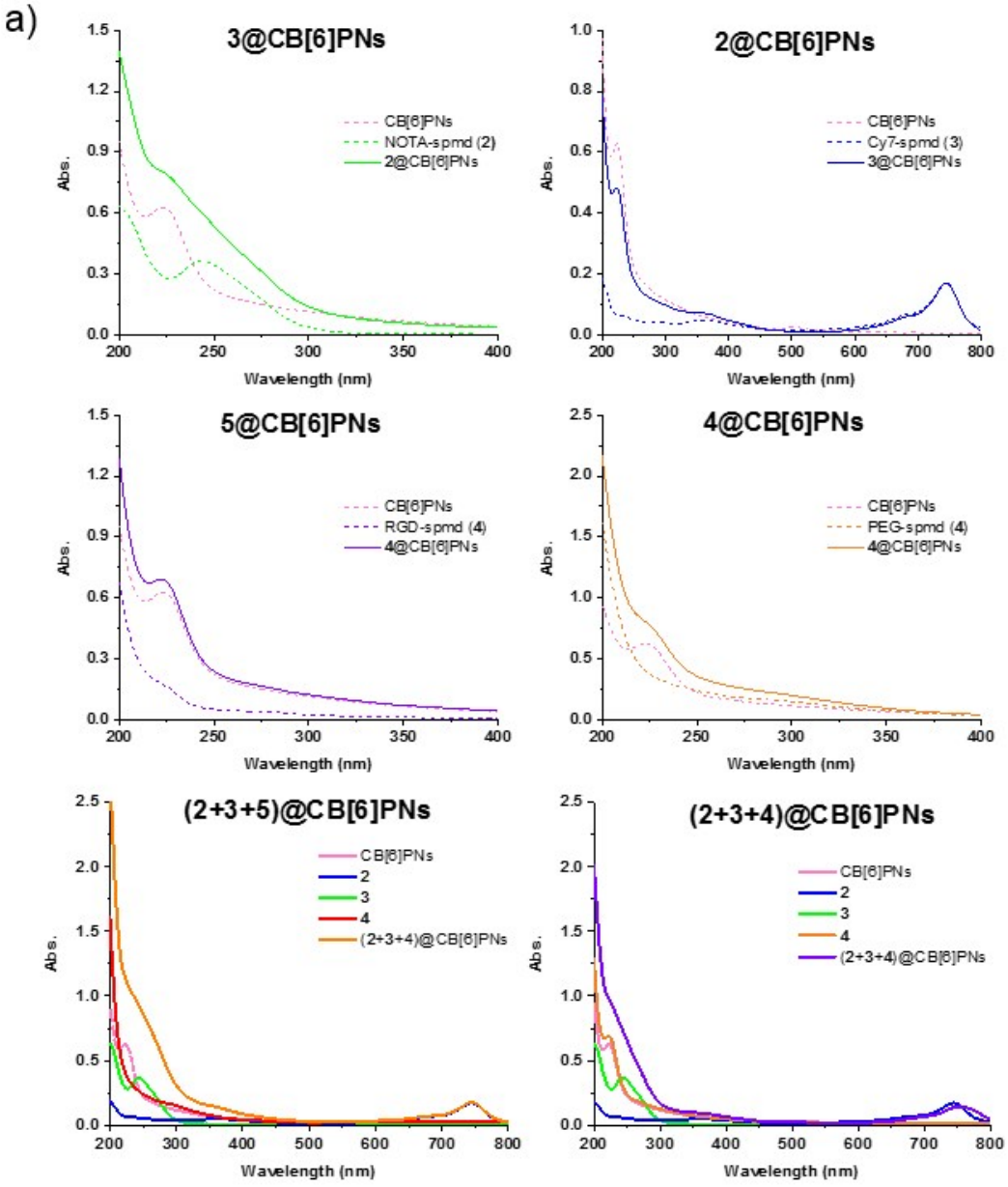


Fig. S11 MALDI-TOF mass spectra of OH-PEG-acetic acid (black) and PEG-spmd (red).

Preparation of 2@CB[6]PNs, 3@CB[6]PNs, 4@CB[6]PNs, 5@CB[6]PNs, (2+3+5)@CB[6]PNs and (2+3+4)@CB[6]PNs: A solution of **2** (20.0 μL from the stock solution of **2**) was added to CB[6]PNs (0.5 mM, 100 μL) in D.I. water to prepare **2**@CB[6]PNs. After incubation for 1 h and dialysis against water for 3 h, the sample was measured by UV-Vis to confirm the existence of **2** in the sample. **2** (20.0 μL from the stock solution of **2**) and **3** (0.2 μL from the stock solution of **3**) was added into **4** (20.0 μL from the stock solution of **4**) or **5** (0.7 μL from the stock solution of **5**) to provide the mixing solution of (**2**+**3**+**4**) or (**2**+**3**+**5**). Following the same procedure for **2**@CB[6]PNs (except with **2**), **3**@CB[6]PNs, **4**@CB[6]PNs, **5**@CB[6]PNs, (**2**+**3**+**4**)@CB[6]PNs and (**2**+**3**+**5**)@CB[6]PNs were also prepared with **3** (0.2 μL from the stock solution of **3**), **4** (20.0 μL from the stock solution of **4**), **5** (0.7 μL from the stock solution of **5**) and a solution of a mixture of (**2**+**3**+**4**) and (**2**+**3**+**5**).

a)



b)

	Size	Zeta potential
CB[6]PNs	180 ± 40 nm	40 ± 8 mV
(**2**+**3**+**5**)@CB[6]PNs	190 ± 70 nm	13 ± 5 mV
(**2**+**3**+**4**)@CB[6]PNs	190 ± 70 nm	14 ± 5 mV

Fig. S12 Characterization of imaging and targeting agents anchored CB[6]PNs by a) UV-Vis in DI water, b) DLS and zeta potential measurements in a serum condition.

Preparation of (2+3), (2+3+5)@CB[6]PNs and (2+3+4)@CB[6]PNs for PET and NIR imaging:

A solution of **2** (20.0 μL from the stock solution of **2**) was added into 3 (170 μCi, 140 μL) in DI water (100 μL) to provide a solution of (2+3). A solution of **2** (40.0 μL from the stock solution of **2**) and **3** (340 μCi, 280 μL) were added into a solution of CB[6]PNs (0.2 mM, 520 μL) and the resulting solution (260 μL) was added into **4** (20.0 μL from the stock solution of **4**) or **5** (0.7 μL from the stock solution of **5**) to provide the mixing solution of (**2**+**3**+**4**)@CB[6]PNs or (**2**+**3**+**5**)@CB[6]PNs.

References:

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