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Mingkang Zhou[a]; Saubhik Haldar[a]; Joseph Franses[a]; Jong-Mok Kim[a]; David H. Thompson[a]
[a] Department of Chemistry, 560 Oval Drive, Purdue University, USA

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Synthesis and Self-assembly Properties of Acylated Cyclodextrins and Nitrilotriacetic Acid (NTA)-modified Inclusion Ligands for Interfacial Protein Crystallization

MINGKANG ZHOU, SAUBHIK HALDAR, JOSEPH FRANSES, JONG-MOK KIM and DAVID H. THOMPSON*

Department of Chemistry, 560 Oval Drive, Purdue University, West Lafayette, IN 47907, USA

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Three different β-cyclodextrins, peracylated on the primary hydroxymethyl rim of the cyclodextrin (6-C_n)$_7$β-CD, and nine different nitrilotriacetic acid-modified inclusion ligands (R-NTA) have been prepared and their self-assembly properties characterized. These modular amphiphiles have been developed to promote two-dimensional crystallization of proteins at the lipid-water interface. Pressure-area isotherm data suggest that the occupancy of the host sites within the (6-C_n)$_7$β-CD monolayers vary as a function of the known host-guest binding constant for the unmodified β-CD host and R guest substituents. Negative-stain TEM experiments show that vesicles are formed upon mild sonication of (6-C_{10})$_7$β-CD and (6-C_{16})$_7$β-CD in 10 mM Tris, pH 7.4 buffer. These results indicate that (6-C_n)$_7$β-CD forms stable lyotropic phases that may be useful for templating the interfacial crystallization of histidine-tagged proteins or other molecules capable of interacting with the R-NTA guest ligands.

Keywords: β-Cyclodextrin; Monolayers; Vesicles; Nitrilotriacetic acid ligands; Protein crystallization

INTRODUCTION

Biological macromolecules are typically conformationally flexible, yet display great specificity with regard to the binding and transformation of their cognate ligands and substrates. A detailed understanding of these molecules at the atomic level, either to decipher structure-property relationships or to design drug candidates that will selectively interact with them, is obtained most directly by examining their crystal structures. Unfortunately, these are not easily obtained, largely due to the lack of predictable and reliable methods for biomacromolecular crystallization.

One method to address this problem, introduced by Uzgiris and Kornberg over 20 years ago using a monoclonal anti-dinitrophenyl (DNP) IgG capable of binding a DNP-lipid [1], is to use the air–water interface to promote two-dimensional (2D) protein crystallization. Through appropriate design of the monolayer, protein present in the subphase will bind to the lipid film and concentrate there in two dimensions (approaching 50–100-fold enrichment in surface concentration). The combined effects of elevated protein concentration, enhanced protein alignment relative to the freely tumbling solution state and fluid monolayer film properties all contribute to the formation of 2D protein crystals. In particular, the presence of a dynamic lipid interface is especially important to afford the bound protein sufficient lateral and rotational mobility to enable self-organization, optimization of intermolecular contacts and efficient packing into a crystalline array [2–5]. The major advantages of this 2D crystallization approach include simple crystal assembly protocols, readily varied monolayer parameters that influence the assembly process and extremely small amounts of protein required for generation and analysis of the 2D crystalline array [6].

Further development of interfacial protein crystallization has generally occurred along two lines of investigation. In some cases, efforts have been directed toward developing new tools for protein crystallization, such as automated methods for analyzing cryopreserved proteins using cryo-transmission electron microscopy (C-TEM) [7], synthesis

*Corresponding author. Tel.: +1-765-494-0386. E-mail: davethom@purdue.edu

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of dialkylglycerol-based compounds with different covalently linked affinity groups [8–10] or adaptation of lipid nanotubes as matrices for helical crystallization and reconstruction of protein structures [11]. Other investigators have focused on using the existing tools to elucidate protein structures from 2D crystals grown on lipid phases. These efforts have led to medium resolution structures (i.e. 10–20 Å) for RNA polymerase A [12], streptavidin [13–18], aminopeptidase M [19], DNA gyrase B [20], HupR [21], Moloney murine leukemia virus capsid [2], HIV-1 virus capsid [22], Annexin V [23] and bacterial S-layer [24].

To date, there are relatively few systematic studies of how the interfacial and solution properties affect the size, morphology and quality of 2D protein crystals. The best-known case is streptavidin, which has been shown to display pH- [16], ionic strength- [18] and amino acid residue-dependent [16,17] crystallization behavior on biotinylated lipid monolayers. Intriguingly, one study comparing streptavidin crystals grown on either biotinylated lipid monolayers or Cu(II)-chelating lipid monolayers found that the crystal morphs were similar, although their gross morphologies were slightly different [15].

This article describes the synthesis and self-assembly properties of a new class of metal-chelating amphiphiles that could enable the systematic study of how interfacial structure and dynamics affect protein crystallization. A key aspect of these amphiphiles is their modular design, based on the well-known noncovalent host-guest interactions between cyclodextrins (CDs) and a potentially vast array of supramolecular complex configurations [25]. The diversity of guest ligands having binding constants (K_{incl}) that vary by as much as 10^4 for various guest–CD pairs underscores the richness of parameter space that is available for promoting 2D protein crystallization [26]. Three different β-CD derivatives, bearing identical C_8, C_{10} or C_{16} acyl substituents on the primary hydroxymethyl rim of the cyclodextrin (i.e. the 6^{A-G}-positions), have been prepared for use as host matrices. Nine different nitrilotriacetic acid (NTA) ligands of varying hydrophobicity and K_{incl} have been synthesized from a common lysine-NTA precursor. Taken together, these noncovalent amphiphiles could enable a new type of lateral diffusion mechanism—i.e. site-hopping—for interrogating the relative importance of solution and interfacial properties for 2D protein crystal growth.

RESULTS AND DISCUSSION

Synthesis of Heptaacyl (6-C_n)$_7$β-CD

Kawabata and coworkers reported the first amphiphilic cyclodextrins, with C_4-, C_8- and C_{12}-chains peralkylated on the hydroxymethyl rim (i.e. the O-6 position) of β-CD [27–30]. Since then, a variety of amphiphilic β-CD derivatives have been reported, bearing alkyl modifications at the O-6 [31], O-2/3 [32,33], and O-6 and O-2/3 [34,35] positions. The syntheses of these and other modified cyclodextrins has been reviewed [36]. In all cases, the amphiphilic character of these compounds arises from the highly water soluble characteristics of the cyclodextrin headgroup (especially in the case of the persulfated derivatives [35]) and the relatively short alkyl chains used.

We have prepared three heptaacyl β-CD derivatives, possessing octanoyl, decanoyl or hexadecanoyl modifications of the 6-βCD hydroxymethyl groups, in 10–36% overall yield using the pathway shown in Fig. 1. These alkyl chains were chosen for the host CD scaffold to confer different phase transition temperatures to their assemblies that may affect protein crystallization. Two of these compounds, (6-C_8)$_7$β-CD (**9**) and (6-C_{10})$_7$β-CD (**8**), were chosen for comparison with the known properties of the octylamine- and dodecylamine-modified CD prepared by Kawabata *et al.*, [27]. The third derivative, (6-C_{16})$_7$β-CD (**7**), was prepared to produce a solid-phase material with very low water solubility. The most significant deviations from reported syntheses of acylated cyclodextrins occurred in the *t*-BuMe_2SiCl O-6 protection and I_2-mediated desilylation steps, where higher yields than those reported were observed.

Synthesis of NTA-modified Guest Ligands for CD Host Complexation

A common lysine-NTA precursor was synthesized as shown in Fig. 2, using an adapted form of our prior synthesis of the Ni^{2+}-chelating lipid, DHGN [2]. The main deviation from the previous synthesis is the use of the methyl ester protected form of lysine (**10**), which enables the use of common organic solvents for subsequent synthetic transformations and chromatographic purification. Intermediate **10** was then used in the coupling reactions with NHS-activated carboxylic acid derivatives (Fig. 3) or sulfonyl chloride derivatives (Fig. 4). Based on the known K_{incl} data for unmodified β-CD and unmodified guest ligands, we anticipate a range of available binding constants from $\sim 10^1$ to $\sim 10^5$ [26], assuming the distal NTA modification has little effect on the host-guest interaction. This expectation is supported by the observed K_{incl} data for β-CD with *trans*-hydroxycinnamic acid ($\sim 10^{2.6}$) and *trans*-hydroxycinnamate ($\sim 10^{2.4}$), wherein the ionizable carboxylic acid substituent is expected to be much closer to the host–guest binding site than would occur for our lysine-NTA guest ligand derivatives. In any case, these guests can be introduced into β-CD assemblies under otherwise identical conditions of ionic strength,

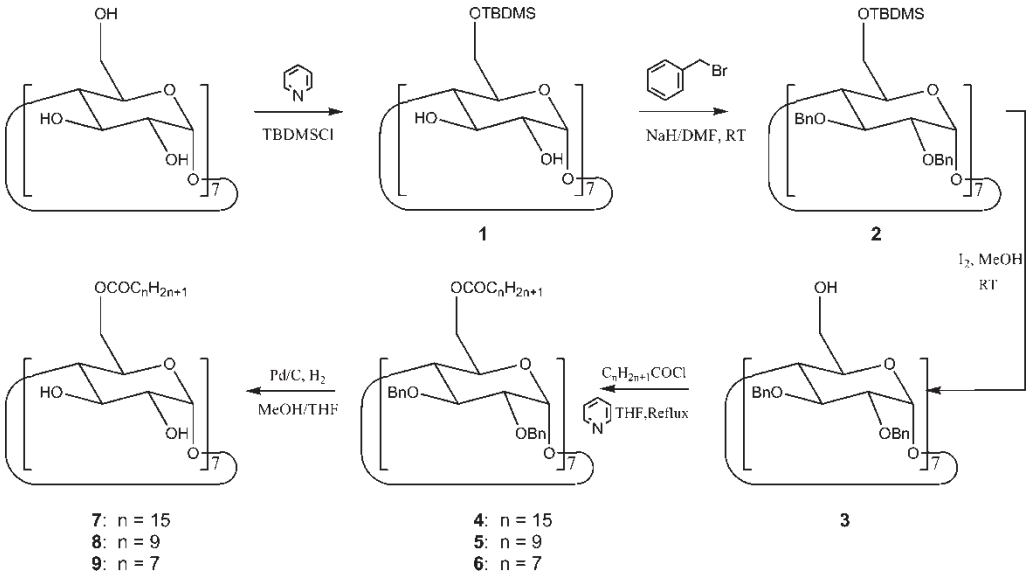

FIGURE 1 Synthesis pathway for $(6\text{-}C_n)_7\beta$-CD derivatives.

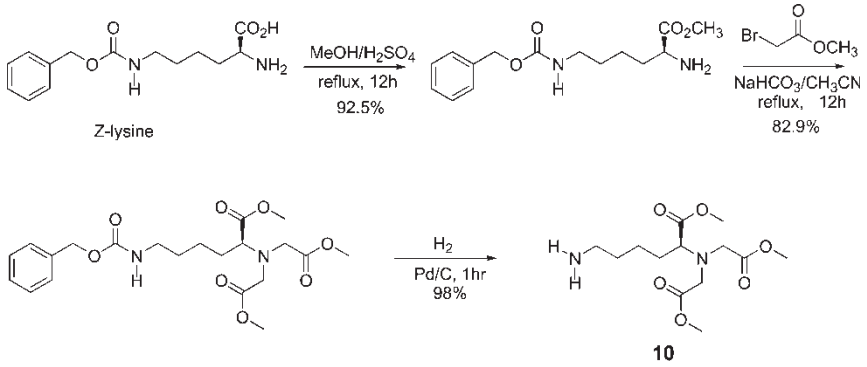

FIGURE 2 Synthesis pathway for N^{α},N^{α}-dicarboxymethyllysine, trimethyl ester.

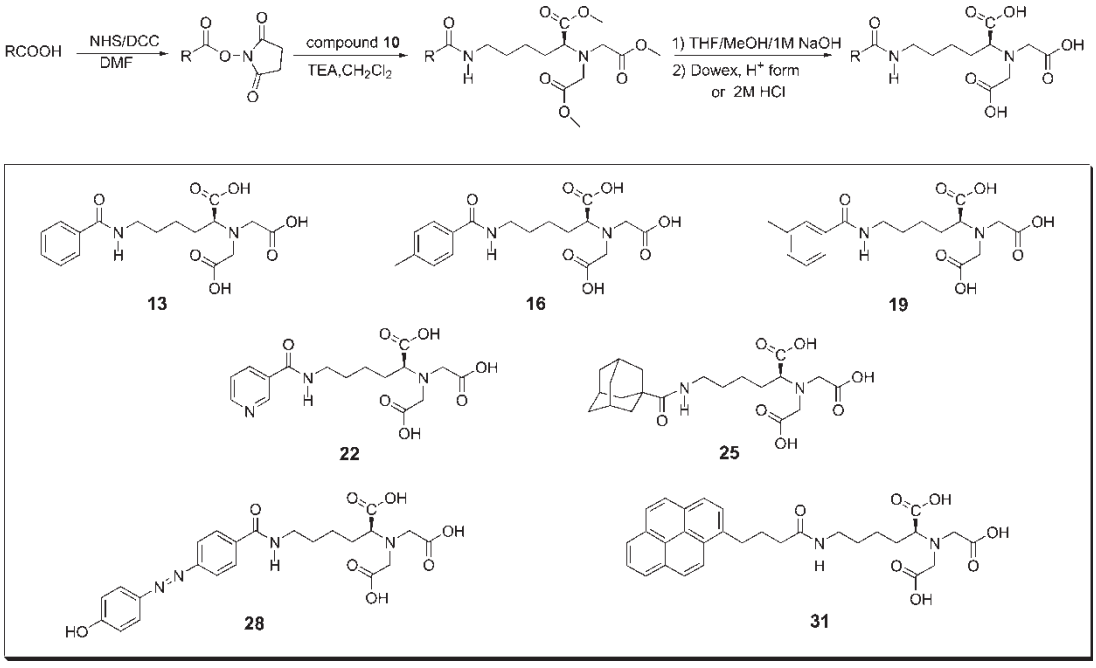

FIGURE 3 *Top*: Synthesis pathway for amide-linked R-NTA derivatives. *Bottom*: Amide-linked NTA guest ligands synthesized using this pathway.

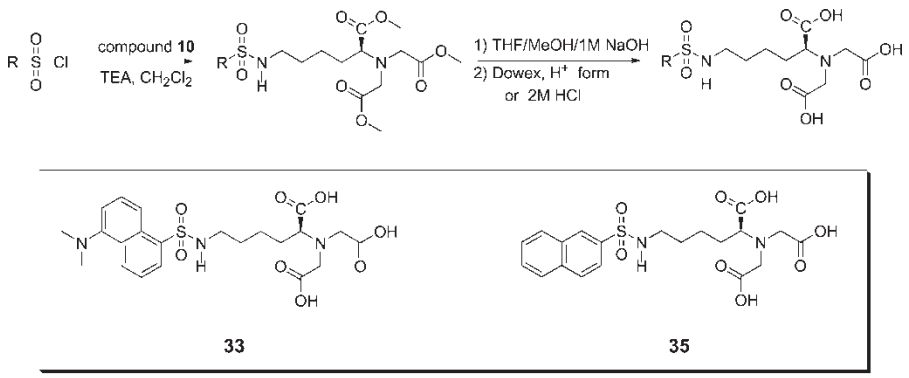

FIGURE 4 *Top*: Synthesis pathway for sulfonamide-linked R-NTA derivatives. *Bottom*: Sulfonamide-linked NTA guest ligands synthesized using this pathway.

pH and temperature, thereby providing direct information on the relative importance of lateral mobility and lattice site occupancy, effects that have been demonstrated by Reinhoudt [37,38] and Craig and colleagues [39] for structurally similar CD derivatives.

Monolayer Behavior of 7 and 8

The pressure–area (π–A) isotherms for the hepta-alkyl-β-CDs **7**, **8** and **9** in the absence and presence of Ad-NTA guest ligand have been determined at the air–water interface at 20°C. The π–A isotherms for **8**, and especially **9**, exhibit low collapse pressures, indicative of unstable monolayer films. The $(6\text{-}C_8)_7$β-CD derivative **9** was not studied further due to monolayer film instability. By contrast, **7** displayed a high collapse pressure (> 45 mM/m) and a limiting molecular area of 205 Å^2 (Fig. 5), parameters that are similar to values reported for other peralkylated CD derivatives ($\beta - \text{CD} = 210$ Å^2) [27]. When a 1:1 mixture of $(6\text{-}C_n)_7$β-CD and NTA guest ligand were dissolved in $CHCl_3$ and spread at the air–water interface, the limiting molecular area of the resulting monolayer increased due to increased electrostatic repulsions between the neighboring $(6\text{-}C_n)_7$β-CD NTA units. In contrast to data reported for azo dye complexation to β-CD monolayers, where the charge on the dye molecule is consant, small, and delocalized [40], complexation of Ni^{2+} to the guest ligand NTA site reduces the overall water solubility of the guest ligand due to charge reduction (2^- → neutral)[†] thereby increasing its affinity for the CD monolayer. This effect was detectable as an increase in the observed limiting molecular area due to increased electrostatic repulsion between neighboring **25**:Ni^{2+} headgroups, arising from a higher occupancy of the host **7** lattice by the adamantane-NTA guest ligands. This interpretation is supported by (1) the observed decrease in limiting molecular area of **7** monolayers in the presence of guest ligand **13** (i.e. phenylcarbonyl-NTA), which should have a lower affinity for the **7** film based on K_{incl} data for unmodified β-CD binding of benzoate derivatives, and (2) the appearance of greater surface pressure at large molecular areas when **25**:Ni^{2+} is used, relative to **13**:Ni^{2+} (e.g. ~ 5 mN/m *vs.* ~ 0 mN/m at 300 Å^2/molecule for **25** and **13**, respectively). Further evidence of monolayer film instability is apparent upon comparison of pure **8** monolayers with **8**:**25** and **8**:**25**:Ni^{2+} monolayers. The observed limiting molecular areas for **8** are larger and have a higher collapse pressure than for the guest complexed films, suggesting that material has been depleted from the interface in the latter cases.

Vesicle Formation via Sonication of 7 and 8

Aqueous dispersions of **7** and **8** were produced by dispersing dried films of $(6\text{-}C_n)_7$β-CD with a probe-type sonicator in the presence of 10 mM Tris, pH 7.4, for 3 min on an ice bath. The films were produced by evaporating an aliquot of a $CHCl_3$ stock solution (50 mM in β-CD) to dryness under a stream of nitrogen gas, prior to addition of the buffer solution. No guest ligand was added to these dispersions. The solution became turbid immediately upon ultrasonification. An aliquot of the suspension was withdrawn by microsyringe and a droplet applied to a TEM grid along with a droplet of $UO_2(OAc)_2$ solution. After blotting away excess UO_2^{2+} staining agent, the samples were imaged by TEM at 5 kV. The data in Fig. 6 show that both **7** and **8** are capable of forming closed membrane structures (i.e. no internal staining of the structures) as would be expected for impermeable vesicles. The capacity of these materials to form dispersed aggregates such as vesicles may be useful for templating the crystallization of proteins for electron tomography and/or automated C-TEM analysis.

[†] Since $pK_{a1} = 1.66$, $pK_{a2} = 2.95$, and $pK_{a3} = 10.2$ for NTA, the uncomplexed form would be a 2^- ion at pH 7.0.

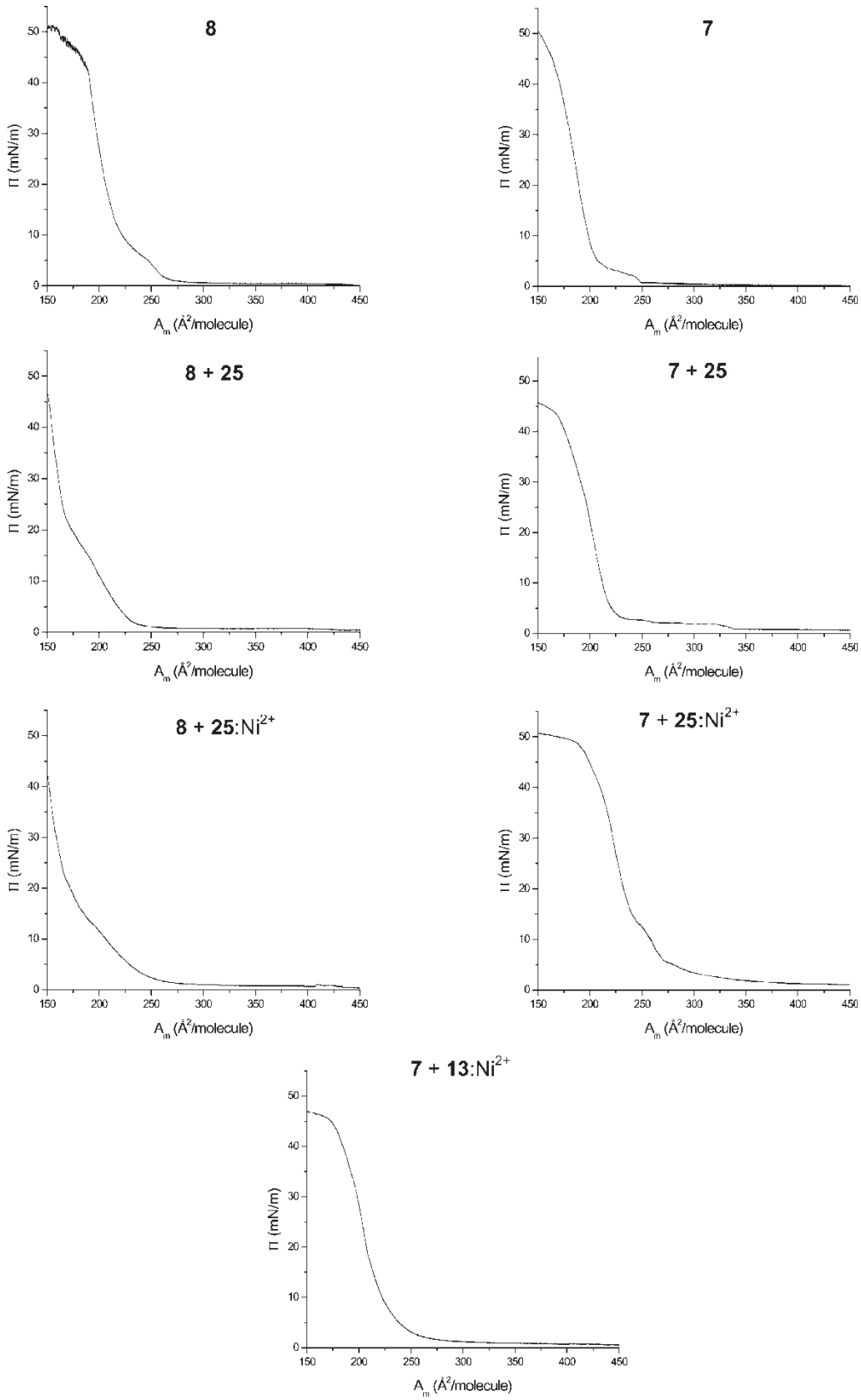

FIGURE 5 Pressure–area isotherms for **8** (*left column*) and **7** (*right column*). *Top row*: (6-C_n)$_7$β-CD alone. *Center row*: (6-C_n)$_7$β-CD in the presence of **25**. *Bottom row*: (6-C_n)$_7$β-CD in the presence of **25**:Ni^{2+}. The monolayer data at the bottom center is for **7** in the presence of **13**:Ni^{2+}.

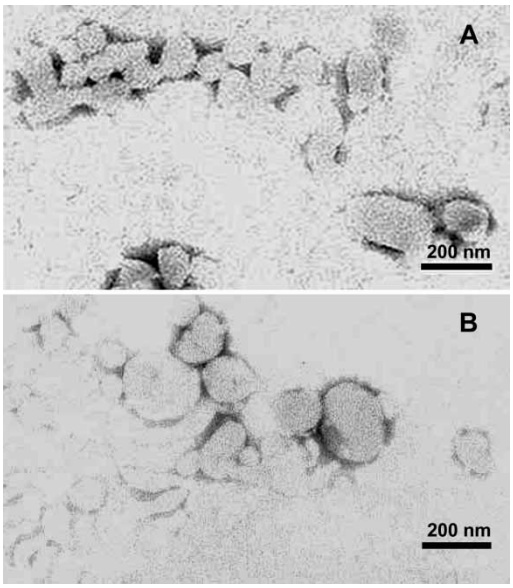

FIGURE 6 Negative-stain TEM images (1% uranyl acetate, poststain) of sonicated dispersions of (a) **8** and (b) **7**. Scale bar = 200 nm.

CONCLUSIONS

We have developed a family of modular amphiphiles, based on O-6 peracylated cyclodextrins and lysine-NTA-modified guest ligands, that are capable of utilizing a site-hopping, rather than lateral lipid diffusion, mechanism for improving the quality of protein crystals grown at the lipid–water interface. Pressure-area isotherm data suggest that the occupancy of the host sites is higher when the guest-NTA K_{incl} is large and when the guest ligand is bound to Ni^{2+}. Monolayer experiments also show that (6-C_n)$_7$β-CD derivatives require longer acyl chains ($> C_{10}$) to produce stable monolayer films. Vesicles are also formed by **7** and **8** when dry films of these (6-C_n)$_7$β-CD derivatives are briefly sonicated. Experiments designed to probe the dynamics of these modular amphiphiles are in progress.

MATERIALS AND METHODS

Synthesis of 6^A,6^B,6^C,6^D,6^E,6^F,6^G-*t*-butyldimethylsilyl-β-cyclodextrin (1)

TBDMSCl (30.4 g, 202 mmol) in 100 mL pyridine was added dropwise to a 200 mL freshly dried pyridine solution of β-cyclodextrin (24.00 g, 21.15 mmol) under an Ar atmosphere at 0–5°C. After the addition was complete, the mixture was warmed to room temperature and stirred for 12 h. The reaction was quenched by pouring the mixture into ice-cold water; the solid generated by this operation was filtered and recrystallized from MeOH:$CHCl_3$ 2–3 times to give **1** (37.8 g, 19.5 mmol) in 92.4% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04 (s, Si(CH_3)$_2$, 6H), 0.88 (s, SiC(CH_3)$_3$, 9H), 3.50–3.78 (m, 4H), 3.91 (d, 1H, J = 9.32), 4.05 (t, 1H, J = 8.7), 4.90 (d, 1H, J = 3.0); ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.12, 18.3, 25.9, 61.6, 72.6, 73.4, 73.6, 81.7, 102.0; MS (MALDI): found 1956 ($M + Na^+$), m/z calcd. for $C_{84}H_{168}O_{35}Si_7$ = 1934.86.

Synthesis of 6^A,6^B,6^C,6^D,6^E,6^F,6-*t*-butyldimethylsilyl-2^A,2^B,2^C,2^D,2^E,2^F,2^G,3^A,3^B,3^C,3^D,3^E,3^F,3^G-Benzyl-β-cyclodextrin (2)

In a three-necked round-bottom flask, 95% NaH (0.41 g, 16.2 mmol) was dissolved in 2 mL of freshly dried DMF and the mixture stirred under an Ar atmosphere at 0–5°C for 15 min. A DMF solution of **1** (0.65 g, 0.336 mmol; in 4 mL DMF) was added dropwise to the NaH solution. After gas evolution ceased, the mixture was allowed to stir for 30 min to assure complete deprotonation of the O-2- and O-3-β-CD hydroxyl groups. Benzyl bromide (1.9 mL, 15.9 mmol) was then added dropwise while maintaining the reaction mixture at 0–5°C. After the addition was complete, the reaction mixture was warmed to room temperature and stirred for 8 h before quenching the reaction through the addition of MeOH at 0–5°C. The reaction mixture was then dissolved in 120 mL pentane, washed with water (3 × 300 mL) and saturated NaCl solution (50 mL). The organic layer was dried over anhydrous Na_2SO_4, evaporated, and purified by silica gel column chromatography using a 10:1–6:4 pentane:CH_2Cl_2 step gradient elution to give compound **2** (0.597 g, 0.186 mmol) in 55.4% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.03 (s, Si(CH_3)$_2$, 6H), 0.89 (s, SiC(CH_3)$_3$, 9H), 3.49 (dd, 1H, J = 2.7, 9.0), 3.69–3.81 (m, 2H), 3.98-4.13 (m, 2H), 4.28 (d, 1H, J = 7.5), 4.48–4.56 (m, 2H), 4.73 (d, 1H, J = 10.8), 5.09 (d, 1H, J = 10.5), 5.33 (d, 1H, J = 2.7), 7.00–7.25 (m, 10H).

Synthesis of 2^A,2^B,2^C,2^D,2^E,2^F,2^G,3^A,3^B,3^C,3^D,3^E,3^F,3^G-benzyl-β-cyclodextrin (3)

I_2 in 11 mL MeOH (1% solution) was added to **2** (0.503 g, 0.157 mmol) in 10 mL of pentane and the solution stirred at room temperature for 7 days. The reaction was quenched by the addition of solid $Na_2S_2O_3$ until the I_2 color disappeared. The colorless solution was then evaporated, redissolved in 100 mL CH_2Cl_2, washed with saturated $NaHCO_3$ (2 × 75 mL) and saturated NaCl solution (2 × 75 mL), before drying over anhydrous Na_2SO_4. Solvent evaporation gave **3** (0.372 g, 0.155 mmol) in 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.60–3.72 (dd, 1 H, J = 2.7, 8.7), 3.81 (t, 1H, J = 7.8), 3.88–4.04 (m, 1H), 4.04–4.20 (m, 3H), 4.58–4.77 (m, 2H), 4.86 (d, 1H, J = 11.1), 5.05 (d, 1H, J = 11.4), 5.21 (s, 1H) 7.22–7.40 (m, 10H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 61.7, 72.7, 72.9, 75.0, 78.4, 78.8, 80.3, 98.2, 127.0, 127.2, 127.4, 127.8, 128.0, 128.1, 138.2, 138.9; MS (MALDI): found 2418 ($M + Na^+$), m/z calcd. for $C_{140}H_{154}O_{35} = 2396.72$.

Synthesis of 6^A,6^B,6^C,6^D,6^E,6^F,6^G-alkanoyl-2^A,2^B,2^C,2^D,2^E,2^F,2^G,3^A,3^B,3^C,3^D,3^E, 3^F,3^G-benzyl-β-cyclodextrin

To an ice-cold mixture of acyl chloride (8.5 mmol) in a mixture of 10 mL dry THF and pyridine (4.2 mL, 52.2 mmol), a 10 mL THF solution of **3** (0.62 g, 0.25 mmol) was added dropwise. After the addition was over (~5 min), the reaction mixture was warmed to room temperature and then heated at reflux with stirring for 17 h in an oil bath. The reaction mixture was then cooled to room temperature, filtered, concentrated, and redissolved in 200 mL CH_2Cl_2. The organic phase was washed with 20% HCl solution (2 × 100 mL), water (100 mL), 15% K_2CO_3 solution (2 × 100 mL), 5% KOH solution (2 × 100 mL), and finally with saturated NaCl solution (2 × 100 mL). The organic phase was dried with anhydrous Na_2SO_4, evaporated, and the residue purified by silica gel column chromatography (hexane wet-packed) using 1:10 EtOAc:hexane as eluent.

Compound **4**: Palmitoyl chloride (1 mL) and **3** (0.504 g, 0.210 mmol) gave **4** (632 mg, 0.155 mmol) in 73.8% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 6.3$) 1.20–1.40 (m, 24H), 1.52–1.70 (m, 2H), 2.29 (t, 2H, $J = 7.5$), 3.46 (dd, 1H, $J = 3.3, 9.3$), 3.76 (d, 1H, $J = 8.7$) 3.95–4.10 (m, 2H), 4.40–4.58 (m, 4H), 4.73 (d, 1H, $J = 12.6$), 4.95–5.05 (m, 2H), 7.08–7.26 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 22.7, 24.9,29.1, 29.2, 29.5, 29.7, 31.9, 34.0, 63.0, 70.0, 72.9, 75.3, 78.8, 79.3, 80.5, 98.6, 127.2, 127.5, 127.8, 128.0, 128.2, 128.4, 138.3, 139.0, 173.0; MS (MALDI): found 4088 ($M + Na^+$), m/z calcd. for $C_{252}H_{364}O_{42} = 4065.61$.

Compound **5**: This was prepared as described above, except that decanoyl chloride was used as acylating agent (~60% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, 3H), 1.20-1.40 (m, 12H), 1.50–1.70 (m, 2H), 2.30 (t, 2H, $J = 7.8$), 3.4–3.51 (m, 1H), 3.77 (t, 1H, $J = 10.0$), 3.95–4.12 (m, 2H), 4.37–4.60 (m, 4H), 4.75 (d, 1H, $J = 11.0$), 4.95–5.08 (m, 2H), 7.10–7.30 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.1, 22.7, 24.9, 29.2, 29.4, 29.5, 29.6, 31.9, 34.0, 63.0, 70.0, 72.9, 75.3, 78.8, 79.4, 80.5, 98.7, 127.1, 127.2, 127.6, 127.5, 128.0, 128.2, 138.4, 139.1, 172.9.

Compound **6**: This was prepared as described above using octanoyl chloride as acylating agent. ^{1}H NMR resonances were identical to those for Compound **5**, except for the integral at 1.2–1.4 ppm, which corresponded to 8H.

Synthesis of 6^A,6^B,6^C,6^D,6^E,6^F,6^G-alkanoyl-β-cyclodextrin

The heptaacyl β-cyclodextrin derivative was dissolved in 10 mL of 1:1 MeOH:THF and the reaction stirred under a H_2 atmosphere in the presence of 10 mg of Pearlman's catalyst for 12 h at room temperature. The reaction mixture was then filtered and the organic phase concentrated to give the desired product.

Compound **7**: Compound **4** (0.356 g) was deprotected to give 0.241 g of **7** (98% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 6.0$) 1.20–1.40 (m, 24H), 1.52–1.70 (m, 2H), 2.22–2.44 (m, 2H), 3.42 (t, 1H, $J = 9.0$), 3.68–3.80 (m, 1H) 3.92–4.16 (m, 3H), 4.53 (d, 1H, $J = 11.4$), 4.90 (s, 1H), 5.22 (s, 1H, —OH), 6.64 (s, 1H, —OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 22.7, 25.0, 29.1, 29.2, 29.4,29.6, 29.7, 29.8, 31.9, 34.0, 62.3, 69.8, 73.2, 73.6, 82.9, 102.0, 173.1; MS (MALDI): found 2826 ($M + Na^+$), m/z calcd. for $C_{154}H_{280}O_{42} = 2803.86$.

Compounds **8** and **9**: These C_{10} and C_8 derivatives, respectively, were prepared as described above and gave comparable analytical data.

Lysine-N^ε-benzylcarbamate, Methyl Ester

Methyl-N^ε-benzylcarbamoyl-lysine ester (23.09 g, 82.36 mmol) was dissolved in 300 mL MeOH containing 10 mL concentrated H_2SO_4 (added dropwise) and the resulting solution heated at reflux for 12 h. After cooling to room temperature, the solution was concentrated, dissolved in 250 mL $CHCl_3$, washed with cold saturated $NaHCO_3$ solution (3 × 50 mL), followed by saturated LiCl solution (3 × 50 mL), and then dried over anhydrous $MgSO_4$. Evaporation of the $CHCl_3$ phase gave a colorless oil (22.43 g, 76.2 mmol, 92.5% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34–1.58 (m, 4H), 1.73–1.95 (m, 2H), 3.13 (m, 2H), 3.42 (t, 1H, $J = 6.0$), 3.70 (s, 3H), 4.90 (s, 1H, NH), 5.08 (s, 2H), 7.25–7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.9, 28.8, 31.4, 40.1, 52.3, 53.0, 66.0, 127.3, 127.6, 128.1, 136.5, 156.3, 172.2; MS (CI): found 295 (MH^+), m/z calcd. for $C_{15}H_{22}N_{2O4} = 294.35$.

N^α,N^α-Dicarboxymethyllysine-N^ε-benzylcarbamate, Trimethyl Ester

Lysine-N^ε-benzylcarbamate, methyl ester (10.05 g, 34.13 mmol) was dissolved in 200 mL dry CH_3CN, along with bromomethyl acetate (16.64 g, 108.8 mmol) and anhydrous $NaHCO_3$ (4 g), and the solution heated at reflux for 12 h. After cooling to room temperature, the solution was concentrated, redissolved in 200 mL Et_2O, washed with saturated NaCl solution (2 × 50 mL) and dried over anhydrous $MgSO_4$. After filtration and solvent

evaporation, the residue was separated by silica gel column chromatography using a hexane:EtOAc step gradient (2:1, then 1:1) to give the desired product (12.4 g, 28.3 mmol) in 82.9% yield. TLC: $R_f = 0.2$ in 2:1 hexane:EtOAc, and $R_f = 0.5$ in 1:1 hexane:EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30–1.60 (m, 4H), 1.60–1.75 (m, 2H), 3.10–3.25 (m, 2H), 3.41 (t, 1H, $J = 7.5$), 3.63 (s, 4H), 3.67 (s, 9H), 4.95 (s, 1H, NH), 5.09 (s, 2H), 7.24–7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.9, 29.3, 29.9, 40.7, 51.3, 51.6, 52.4, 64.5, 66.4, 127.9, 128.4, 136.7, 156.4, 171.7, 173.0; MS (CI): found 439 (MH^+), m/z calcd. for $C_{21}H_{30}N_2O_8 = 438.47$.

N^{α},N^{α}-Dicarboxymethyllysine, Trimethyl Ester (10)

Palladium on carbon catalyst (~10 mg of 10 wt% Pd/C, Degussa type E101) was added in MeOH with N^{α},N^{α}-dicarboxymethyllysine-N^{ε}-benzylcarbamate, trimethyl ester (1.00 g, 2.28 mmol) and a balloon filled with H_2 attached to the top of the air-tight flask. The reaction mixture was stirred for 1 h before processing by filtration and evaporation to give **10** (0.680 g, 2.23 mmol) in 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40–1.82 (m, 6H, 2H + 4H), 2.94 (t, 2H, $J = 7.5$), 3.50 (t, 1H, $J = 7.5$), 3.68 (s, 4H), 3.74 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.8, 29.1, 29.8, 40.4, 51.4, 51.7, 52.4, 64.4, 171.9, 173.0; MS (CI): found 305 (MH^+), m/z calcd. for $C_{13}H_{24}N_2O_6 = 304.34$.

Synthesis of NHS Esters of Carboxylic Acid Guest Ligand Intermediates

One equivalent each of *N*-hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC), and the carboxylic acid derivative were dissolved in 50 mL DMF and the solution stirred for 12 h. The reaction mixture was processed by DCU byproduct removal via filtration, DMF evaporation and recrystallization of the residue from CH_2Cl_2:hexane to give the purified NHS ester of the corresponding carboxylic acid.

Compound **11**: Benzoic acid (1.033 g, 8.46 mmol), NHS (0.974 g, 8.46 mmol), and DCC (1.745 g, 8.46 mmol) gave **11** (1.430 g, 6.52 mmol) in 77.1% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.89 (s, 4H), 7.51 (t, 2H, $J = 7.8$), 7.67 (t, 1H, $J = 7.2$), 8.13 (d, 2H, $J = 8.1$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.1, 124.6, 128.3, 130.0, 134.3, 161.0, 168.4; MS (CI): found 220 (MH^+), m/z calcd. for $C_{11}H_9NO_4 = 219.19$.

Compound **14**: *p*-Toluic acid (1.008 g, 7.404 mmol), NHS (0.852 g, 7.402 mmol), and DCC (1.528 g, 7.406 mmol) gave **14** (1.564 g, 6.706 mmol) in 90.6% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.45 (s, 3H), 2.90 (s, 4H), 7.31 (d, 2H, $J = 8.1$), 8.03 (d, 2H, $J = 8.1$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.7, 25.5, 122.1, 129.4, 130.4, 146.0, 161.8, 169.3; MS (CI): found 234 (MH^+), m/z calcd. for $C_{12}H_{11}N_2O_4 = 233.22$.

Compound **17**: *m*-Toluic acid (3 g, 22.03 mmol), NHS (2.536 g, 22.04 mmol), and DCC (4.546 g, 22.03 mmol) gave **17** (3.856 g, 16.534 mmol) in 75% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.43 (s, 3H), 2.91 (s, 4H), 7.40 (t, 1H, $J = 8.1$), 7.49 (d, 1H, $J = 4.5$), 7.92–8.00 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.7, 26.1, 124.5, 127.2, 128.2, 130.4, 135.1, 138.2, 161.2, 168.4; MS (CI): found (MH^+) 234, m/z calcd. for $C_{12}H_{11}NO_4 = 233.22$.

Compound **20**: Nicotinic acid (3 g, 24.37 mmol), NHS (2.805 g, 24.37 mmol), and DCC (5.028 g, 24.37 mmol) gave **20** (4.612 g, 20.95 mmol) in 86% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.93 (s, 4H), 7.44–7.53 (m, 1H), 8.41 (dd, 1H, $J = 1.8$, 6.0), 8.89 (d, 1H, $J = 3.6$), 9.34 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$ 2185): δ 26.1, 121.3, 123.3, 137.4, 150.6, 154.3, 160.0, 168.0; MS (CI): found 221 (MH^+), m/z calcd. for $C_{10}H_8N_2O_4 = 220.18$.

Compound **23**: Adamantane-1-carboxylic acid (4.690 g, 26.02 mmol), NHS (2.995 g, 26.02 mmol), and DCC (5.369 g, 26.02 mmol) gave **23** (4.901 g, 17.67 mmol) in 67.9% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78 (s, 6H), 2.10 (s, 9H), 2.84 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.6, 27.6, 36.1, 38.4, 40.5, 169.2, 172.3; MS (CI): found 279 (MH^+), m/z calcd. for $C_{15}H_{19}NO_4 = 277.32$.

Compound **26**: 4-(4-Hydroxyphenylazo)benzoic acid (2.422 g, 10 mmol), NHS (1.151 g, 10 mmol), and DCC (2.063 g, 10 mmol) gave **26** (3.042 g, 8.97 mmol) in 89.7% yield after recrystallization from acetone:hexane. ^{1}H NMR (300 MHz, d_6-DMSO): δ 2.97 (s, 4H), 7.03 (d, 2H, $J = 8.4$), 7.94 (d, 2H, $J = 8.7$), 8.05 (d, 2H, $J = 8.4$), 8.32 (d, 2H, $J = 8.4$); ^{13}C NMR (75 MHz, d_6-DMSO): δ 25.6, 116.2, 122.8, 125.2, 125.7, 131.5, 145.4, 156.0, 161.4, 162.2, 170.2; MS (CI): found 340 (MH^+), m/z calcd. for $C_{17}H_{13}N_3O_5 = 339.3$.

Synthesis of Trimethyl Ester-protected Lysine-N^{ε}-carboxamide Guest Ligand Intermediates

One equivalent each of Compound **10** and the NHS ester of the corresponding carboxylic acid derivative were combined in 100 mL CH_2Cl_2 with 1 mL Et_3N and the reaction mixture stirred for 12 h at room temperature. The solvent was then evaporated, the residue redissolved in $CHCl_3$, and the organic layer dried with saturated NaCl solution (3 × 50 mL) and anhydrous Na_2SO_4. After filtration and evaporation, the residue was purified by silica gel column chromatography using 1:1 hexane:EtOAc as eluent to give the desired NTA trimethyl ester.

Compound **12**: Compounds **11** (0.450 g, 2.05 mmol) and **10** (0.625 g, 2.05 mmol) gave **12** (408 mg, 0.999 mmol) in 48.7% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44–1.80 (m, 6H), 3.38–3.50 (m, 3H),

3.57–3.75 (m, 13H), 6.52–6.59 (m, 1H), 7.35–7.50 (m, 3H), 7.81 (d, 2H, $J = 7.8$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.9, 28.5, 29.7, 39.7, 51.4, 51.6, 52.5, 64.3, 127.0, 128.3, 131.1, 134.8, 167.6, 171.8, 173.1; MS (CI): found 409 (MH^+), m/z calcd. for $C_{20}H_{28}N_2O_7 = 408.45$.

Compound **15**: Compounds **14** (0.309 g, 1.325 mmol) and **10** (0.403 g, 1.324 mmol) gave **15** (311 mg, 0.736 mmol) in 55.6% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40–1.78 (m, 6H), 2.35 (s, 3H), 3.35–3.50 (m, 3H), 3.55–3.72 (m, 13H), 6.50–6.64 (m, 1H, NH), 7.18 (d, 2H, $J = 8.1$), 7.69 (d, 2H, $J = 8.1$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.3, 22.9, 28.6, 29.6, 39.5, 51.3, 51.5, 52.5, 64.3, 126.9, 129.0, 131.9, 141.4, 167.5, 171.8, 173.0; MS (CI): found 423 (MH^+), m/z calcd. for $C_{21}H_{30}N_2O_7 = 422.47$.

Compound **18**: Compounds **17** (0.541 g, 2.32 mmol) and **10** (0.706 g, 2.32 mmol) gave **18** (0.665 g, 1.57 mmol) in 67.8% yield. TLC: $R_f = 0.25$, 1:1 hexane:EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44–1.84 (m, 6H), 2.44 (s, 3H), 3.43–3.56 (m, 3H), 3.67 (s, 3H), 3.70 (s, 6H), 3.73 (s, 4H), 6.55 (s, 1H, NH), 7.30–7.40 (m, 2H), 7.60–7.65 (m, 1H), 7.69 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1, 22.9, 28.5, 29.6, 39.5, 51.3, 51.5, 52.4, 64.3, 123.9, 127.6, 128.1, 131.7, 134.7, 138.0, 167.7, 171.7, 173.0; MS (positive ESI): found 423 (MH^+), m/z calcd. for $C_{21}H_{30}N_2O_7 = 422.47$.

Compound **21**: Compounds **20** (0.512 g, 2.32 mmol) and **10** (0.708 g, 2.33 mmol) gave **21** (0.701 g, 1.71 mmol) in 73.6% yield after silica gel column chromatography using 10:1 EtOAc:MeOH as eluent. TLC: $R_f = 0.25$, 10:1 EtOAc:MeOH. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50–1.80 (m, 6H), 3.43–3.56 (m, 3H), 3.62 (s, 3H), 3.65 (s, 6H), 3.69 (s, 4H), 7.00 (s, 1H, NH), 7.43 (m, 1H), 8.26 (d, 1H, $J = 8.1$), 8.71 (dd, 1H, $J = 1.5, 4.8$), 9.12 (d, 1H, $J = 1.8$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.6, 27.9, 29.3, 39.6, 51.2, 51.4, 52.4, 63.9, 123.1, 130.4, 135.2, 147.9, 151.3, 165.6, 171.7, 172.9; MS (positive ESI): found 410 (MH^+), m/z calcd. for $C_{19}H_{27}N_3O_7 = 409.43$.

Compound **24**: Compounds **23** (0.847 g, 3.42 mmol) and **10** (0.955 g, 3.14 mmol) gave **24** (0.820 g, 2.003 mmol) in 58.7% yield after column chromatography with 20:1 $CHCl_3$:MeOH. TLC: $R_f = 0.5$, 20:1 $CHCl_3$:MeOH. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35–1.60 (m, 4H), 1.64–1.79 (m, 8H, 6H + 2H), 1.80–1.88 (m, 6H), 2.03 (s, 3H), 3.18–3.30 (m, 2H), 3.41 (t, 1H, $J = 7.2$), 3.63 (s, 4H), 3.68 (s, 9H), 5.71 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.1, 28.2, 29.0, 30.0, 36.6, 39.0, 39.3, 40.6, 51.5, 51.7, 52.5, 64.6, 171.9, 173.1, 178.0; MS (positive ESI): found 467 (MH^+), m/z calcd. for $C_{24}H_{38}N_2O_7 = 466.57$.

Compound **27**: Compounds **26** (1.512 g, 4.46 mmol) and **10** (1.356 g, 4.46 mmol) gave **27** (1.54 g, 2.91 mmol) in 65.4% yield. TLC: $R_f = 0.35$, 2:1 hexane:EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44–1.84 (m, 6H), 3.43–3.56 (m, 3H), 3.63 (s, 3H), 3.65 (s, 6H), 3.68 (s, 4H), 6.69 (d, 2H, $J = 8.7$), 7.80–7.90 (m, 4H), 7.90–8.00 (d, 2H, $J = 8.1$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.9, 28.2, 29.5, 40.0, 51.5, 51.7, 52.7, 64.2, 116.0, 122.4, 125.2, 128.0, 135.3, 146.5, 154.5, 160.4, 167.8, 172.1, 173.3; MS (positive ESI): found 529 (MH^+), m/z calcd. for $C_{26}H_{32}N_4O_8 = 528.55$.

Compound **30**: Compounds **29** (1.034 g, 2.68 mmol) and **10** (0.817 g, 2.68 mmol) gave **30** (0.691 g, 1.20 mmol) in 44.8% yield. TLC: $R_f = 0.25$, 2:1 hexane:EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30–1.56 (m, 4H), 1.56–1.70 (m, 2H), 2.05–2.35 (m, 4H), 3.12–3.30 (m, 2H), 3.30–3.45 (m, 3H), 3.46–3.70 (m, 13H), 5.91 (s, 1H, NH), 7.83 (d, 1H, $J = 7.5$), 7.88–8.20 (m, 7H), 8.28 (d, 1H, $J = 9.0$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.3, 26.9, 28.0, 29.0, 32.1, 35.1, 38.4, 50.6, 50.8, 51.7, 63.6, 122.7, 124.0, 124.1, 125.1, 125.8, 126.5, 126.7, 128.0, 129.0, 130.1, 130.6, 135.4, 171.1, 172.3; MS (positive ESI): found 575 (MH^+), m/z calcd. for $C_{33}H_{38}N_2O_7 = 574.66$.

Synthesis of N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-carboxamides (NTA Amide Guest Ligands)

The corresponding trimethyl ester-protected lysine-N^{ε}-carboxamide guest ligand intermediate was dissolved in 12 mL THF, 4 mL MeOH and 4 mL 1 M NaOH and the solution stirred until TLC analysis indicated that all trimethyl ester starting material was consumed (typically, 2 h reaction time). Dowex 50WX8 resin (50–100 mesh, H^+ form) was then added until the pH was below 3. The reaction mixture was then filtered, concentrated, and lyophilized to give the acid form of the desired NTA guest ligand.

N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-benzoamide, **13**: Compound **12** (408 mg, 0.999 mmol) was hydrolyzed to give **13** (254 mg, 0.693 mmol) in 69.4% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.40–1.65 (m, 4H), 1.80–2.00 (m, 2H), 3.29 (t, 2H, $J = 6.3$), 4.05–4.20 (m, 5H), 7.40 (t, 2H, $J = 7.5$), 7.49 (d, 1H, $J = 7.5$), 7.63 (d, 2H, $J = 6.9$); ^{13}C NMR (75 MHz, CD_3OD): δ 24.8, 28.9, 30.1, 40.5, 55.3, 67.8, 128.4, 129.7, 132.7, 135.8, 170.5, 171.3, 172.3; MS (positive ESI): found 367 (MH^+), m/z calcd. for $C_{17}H_{22}N_2O_7 = 366.37$.

N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-4′-methylbenzoamide, **16**: Compound **15** (311 mg, 0.736 mmol) was hydrolyzed to give **16** (0.209 g, 0.549 mmol) in 74.6% yield. ^{1}H NMR (300 MHz, CD_3OD): δ 1.40–1.90 (m, 6H), 2.38 (s, 3H), 3.37 (t, 2H, $J = 6.3$), 3.48 (t, 1H, $J = 6.9$), 3.58–3.72 (m, 4H), 7.25 (d, 2H, $J = 7.8$), 7.70 (d, 2H, $J = 8.1$); ^{13}C NMR (75 MHz, CD_3OD): δ 21.6, 25.0, 30.2, 30.8, 40.8, 55.5, 66.8, 128.4, 130.2, 133.1, 143.3, 170.4, 176.0; MS (positive ESI): found 381 (MH^+), m/z calcd. for $C_{18}H_{24}N_2O_7 = 380.39$.

N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-3′-methylbenzoamide, **19**: Compound **18** (0.665 , 1.57) was hydrolyzed

to give **19** (0.516 g, 1.36 mmol) in 86.2% yield. ^{1}H NMR (300 MHz, CD_3OD): δ 1.40–1.78 (m, 4H), 1.80–2.00 (m,2H), 2.30–2.44 (m, 3H), 3.30–3.46 (m, 2H), 3.90–4.02 (m, 1H), 4.02–4.14 (m, 4H), 7.25–7.40 (m, 2H), 7.55–7.70 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ 21.2, 24.4, 29.5, 30.0, 40.5, 54.5, 67.3, 125.5, 128.9, 129.6, 133.4, 135.7, 139.6, 170.6, 171.0, 172.1; MS (positive ESI): found 381 (MH^+), m/z calcd. for $C_{18}H_{24}N_2O_7 = 380.39$.

N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-3′-nicotinamide, **22**: Compound **21** (700 mg, 1.71 mmol) was hydrolyzed to give **22** (0.422 g, 1.15 mmol) in 67.2% yield. ^{1}H NMR (300 MHz, D_2O): δ1.40–1.75 (m, 4H), 1.80–2.00 (m, 2H), 3.40 (t, 2H, $J = 6.0$), 3.82–3.98 (m, 5H), 8.16 (t, 1H, $J = 6.3$), 8.90 (t, 2H, $J = 8.4$), 9.13 (s, 1H); ^{13}C NMR (75 MHz, D_2O): δ 23.5, 26.4, 27.9, 39.8, 55.2, 67.8, 127.7, 133.6, 140.9, 143.5, 145.1, 164.3, 170.2, 172.2; MS (positive ESI): found 368 (MH^+), m/z calcd. for $C_{16}H_{21}N_3O_7 = 367.35$.

N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-1′-adamantanecarboxamide, **25**: Compound **24** (0.820 g, 1.76 mmol) was hydrolyzed to give **25** (478 mg, 1.126 mmol) in 64% yield. ^{1}H NMR (300 MHz, CD_3OD): δ 1.24–1.60 (m, 4H), 1.60–1.90 (m, 14H), 1.90–2.10 (m, 3H), 3.04–3.22 (br, 2H), 3.37–3.50 (m, 1H), 3.50–3.70 (m, 4H), 7.22–7.42 (s, 1H, NH); ^{13}C NMR (75 MHz, CD_3OD): δ 24.9, 29.8, 30.2, 30.9, 37.8, 40.2, 40.3, 42.0, 55.5, 66.9, 176.0, 181.0; MS (positive ESI): found 425 (MH^+), m/z calcd. for $C_{21}H_{32}N_2O_7 = 424.49$.

N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-4′-(4″-hydroxyphenylazo)benzoamide, **28**: Compound **27** (1.045 g, 1.98 mmol) was hydrolyzed and the product isolated by acidification with 2 M HCl, added dropwise until the pH was <2, to give an orange precipitate of **28** (801 mg, 1.65 mmol, 83.3% yield). ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.44–1.95 (m, 6H), 3.42 (t, 2H, $J = 6.6$), 3.50 (t, 1H, $J = 6.9$), 3.60–3.70 (m, 4H), 6.93 (d, 2H, $J = 9.0$), 7.80–8.00 (m, 6H); ^{13}C NMR (75 MHz, d_6-DMSO): δ 25.0, 30.1, 30.8, 40.1, 55.5, 66.8, 117.1, 123.4, 126.5, 129.4, 137.0, 147.7, 156.0, 162.8, 169.7, 176.0; MS (positive ESI): found 487 (MH^+), m/z calcd. for $C_{23}H_{26}N_4O_8 = 486.47$.

N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-1′-pyrene-4″-butanamide, **31**: Compound **30** (0.464 g, 0.807 mmol) was hydrolyzed and the product isolated by acidification with 2HCl, added dropwise until the pH was <3, to give **31** as a light tan precipitate (379 mg, 0.712 mmol, 88.2% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 1.30–1.85 (m, 6H), 2.00–2.20 (br, 2H), 2.20–2.40 (br, 2H), 3.14 (s, 2H), 3.20–3.35 (m, 2H), 3.35–3.50 (m, 1H), 3.50–3.70 (m, 4H), 7.81 (d, 1H, $J = 5.1$), 7.85–7.96 (m, 3H), 7.96–8.16 (m, 4H), 8.23 (d, 1H, $J = 9.0$); ^{13}C NMR (75 MHz, CD_3OD): δ 24.9, 29.1, 30.0, 30.8, 33.9, 36.9, 40.3, 55.4, 66.8, 124.5, 125.9, 126.0, 126.2, 126.3, 127.0, 127.7, 128.4, 128.5, 128.6, 130.0, 131.4, 132.4, 132.9, 137.4, 176.0; MS (positive ESI): found 533 (MH^+), m/z calcd. for $C_{30}H_{32}N_2O_7 = 532.58$.

Synthesis of Trimethyl Ester-protected Sulfonamide-linked NTA Guest Ligand Intermediates

One equivalent each of the corresponding sulfonyl chloride and **10** were dissolved in 100 mL CH_2Cl_2 with 1 mL of Et_3N. The mixture was stirred for 12 h, then evaporated, redissolved in 200 mL $CHCl_3$, extracted with saturated NaCl solution (3 × 50 mL), and dried over anhydrous $MgSO_4$. This solution was then filtered, evaporated, and the residue purified by silica gel column chromato graphy using 2:1 hexane:EtOAc as eluent.

Compound **32**: Dansyl chloride (0.833 g, 3.09 mmol) and **10** (0.939 g, 3.09 mmol) gave **32** (0.963 g, 1.79 mmol) in 57.9% yield. TLC: $R_f = 0.2$, 2:1 hexane:EtOAc. ^{1}H NMR (300 MHz $CDCl_3$): δ 1.20–1.56 (m, 6H, 4H + 2H), 2.83 (s, 6H), 2.86 (m, 2H), 3.24 (t, 1H, $J = 7.5$), 3.54 (s, 4H), 3.60 (s, 3H), 3.62 (s, 6H), 5.30 (t, 1H, NH, $J = 6.0$), 7.13 (d, 1H, $J = 7.5$), 7.48 (q, 2H, $J = 7.5$), 8.19 (d, 1H, $J = 7.2$), 8.30 (d, 1H, $J = 8.4$), 8.48 (d, 1H, $J = 8.4$); ^{13}C NMR (75 , $CDCl_3$): δ 22.4, 28.7, 29.3, 42.7, 45.1, 51.2, 51.4, 52.2, 64.1, 114.9, 118.7, 122.9, 128.0, 129.2, 129.4, 129.7, 130.0, 134.9, 151.7, 171.6, 172.7; MS (positive ESI): found 538 (MH^+), m/z calcd. for $C_{25}H_{35}N_3O_8S = 537.63$.

Compound **34**: 2-Naphthalenesulfonyl chloride (0.588 g, 2.59 mmol) and **10** (0.789 g, 2.59 mmol) gave **34** (0.919 g, 1.86 mmol) in 71.8% yield. TLC: $R_f = 0.2$, 2:1 hexane:EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37–1.57 (m, 4H), 1.57–1.68 (m, 2H), 3.01 (m, 2H), 3.37 (t, 1H, $J = 7.5$), 3.58–3.62 (m, 4H), 3.66 (s, 3H), 3.69 (s, 6H), 4.90 (t, 1H, NH, $J = 5.7$), 7.63 (m, 2H), 7.81–8.00 (m, 4H), 8.45 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.5, 28.7, 29.4, 42.8, 51.3, 51.5, 52.4, 64.2, 122.3, 127.3, 127.7, 128.2, 128.5, 129.1, 129.3, 132.0, 134.6, 136.8, 171.7, 172.9; MS (positive ESI): found 495 (MH^+), m/z calcd. for $C_{23}H_{30}N_2O_8S = 494.56$.

Synthesis of N^{α},N^{α}-Dicarboxymethyllysine-N^{ε}-sulfonamides (NTA Sulfonamide Guest Ligands)

The corresponding trimethyl ester-protected lysine-N^{ε}-sulfonamide guest ligand intermediate was dissolved in 12 mL THF, 4 mL MeOH and 4 mL 1 M NaOH and the solution stirred until TLC analysis indicated that all trimethyl ester starting material was consumed (typically, 2 h reaction time). Dowex 50WX8 resin (50–100 mesh, H^+ form) was then added until the pH was below 3. The reaction mixture was then filtered, concentrated and lyophilized to give the acid form of the desired NTA guest ligand.

Compound **33**: Compound **32** (527 mg, 0.98 mmol) was hydrolyzed to give **33** (0.319 g, 0.644 mmol) in 65.7% yield. ^{1}H NMR (300 MHz, D_2O): δ 1.29 (br s, 4H), 1.74 (br s, 2H), 2.88 (br s, 2H), 3.59 (br s, 6H), 3.96 (br s, 1H), 4.25 (br s, 4H), 7.89 (br s, 2H), 8.16 (br s, 1H),

8.23 (br s, 1H), 8.54 (br s, 1H), 8.71 (br s, 1H); ^{13}C NMR (75 MHz, D_2O): δ 22.7, 26.3, 28.1, 42.2, 47.2, 54.4, 66.8, 119.9, 125.5, 125.8, 126.7, 127.2, 128.3, 128.5, 130.4, 135.4, 138.4, 169.5, 170.6; MS (positive ESI): found 496 (MH^+), m/z calcd. for $C_{22}H_{29}N_3O_8S = 495.55$.

Compound **35**: Compound **34** (532 mg, 1.08 mmol) was hydrolyzed and the product isolated by acidification with 2 M HCl, added dropwise, to give **35** as a white precipitate. The precipitate was redissolved and reprecipitated with water two more times to give 353 mg (0.780 mmol) of product in 72.4% yield. Compound **35** is soluble in MeOH and acetone, but not $CHCl_3$ or water. ^{1}H NMR (300 MHz, CD_3OD): δ 1.30–1.78 (m, 6H), 2.82–2.96 (m, 2H), 3.30–3.45 (m, 1H), 3.50–3.65 (m, 4H), 7.55–7.70 (m, 2H), 7.85 (d, 1H, $J = 8.7$), 7.95 (d, 1H, $J = 7.5$), 7.98–8.10 (m, 2H), 8.41 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 24.6, 30.3, 30.5, 43.9, 55.4, 66.7, 123.6, 128.8, 129.1, 129.9, 130.3, 130.6, 133.7, 136.2, 138.9, 176.0; MS (positive ESI): found 453 (MH^+), m/z calcd. for $C_{20}H_{24}N_2O_8S = 452.48$.

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