highly helical C peptides based on C34 proved to be a useful guide for developing highly active fusion inhibitors.

In this work we have developed soluble and highly active inhibitors of the fusion of HIV-1 with target cells based on the remodeling of a parent C34 pharmacophore by introducing *i* to *i* + 4 Glu-Lys pairs into the solvent-accessible surface of the six-helix bundle. Effects on anti-HIV-1 activity of amino acid changes in the inner-strand contact surface have been well documented.[4e,7b] However, little attention has been paid to the effects of substitution in the outer surface of the six-helix bundle.[7a] The α-helical SC peptides reported herein can be divided functionally into two α-helical surfaces: surfaces that interact with the inner strand where Z residues (*a*, *d*, and *e* in Figure 1 B) are located, and surfaces that are responsible for α-helix formation, which are formed by both Glu and Lys residues. Reportedly, the latter is needed for appropriate positioning of Z residues through α-helix formation. Therefore, other approaches for presenting Z functionality potentially could be used as alternatives to replacement with Glu-Lys pairs.[9] A Z-EE-ZZ-KK repeated peptide such as **3** could serve as a scaffold to explore the interactive surfaces responsible for anti-HIV-1 activity. Finally, we have elucidated the structures of N36/SC peptide complexes using X-ray analysis in order to finetune the Z residues.

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Structure–Function Analysis of Urotensin II and Its Use in the Construction of a Ligand–Receptor Working Model

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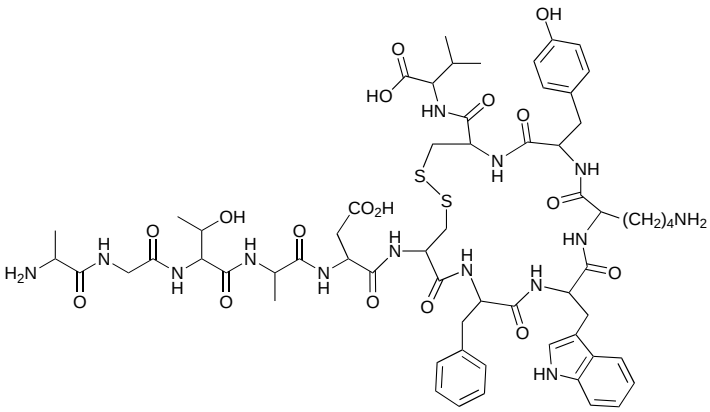
Urotensin II (U II) is a cysteine-linked cyclic peptide with potent vasoactive properties. Originally, this substance was isolated from the urophysis (a caudal neurosecretory organ) of the goby fish (*Gillichthys mirabilis*) as a 12-mer, AG-TADCFWKYCV (Scheme 1),[1] but it has now been identified



Scheme 1. U II peptide from the goby fish (**1**).

in all classes of vertebrates. The composition of U II ranges from 11 amino acids in humans to 14 amino acids in mice, always with the conserved cysteine-linked macrocycle, CFWKYC. Recently, the U II receptor was identified[2] as a G-protein-coupled receptor (GPCR) previously known as the GPR14 orphan receptor,[3] which is expressed predominantly in cardiovascular tissues.

Goby U II (**1**) possesses powerful vasoconstrictor activity in fish, mammals, and humans.[4,5] Moreover, it appears to be the most potent vasoconstrictor known,[6] causing concentration-dependent contraction of isolated arterial rings of rats and humans with an EC_{50} value of less than 1 nM, which means it is about ten times more potent than endothelin 1. However, the

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in vivo effects of U II can be tissue- and species-specific, and sometimes contradictory. For example, U II has also been described as a vasodilator in conscious rats.[7] In addition, this peptide was recently reported to induce hypertrophy in cardiomyocytes[8] and the proliferation of smooth muscle cells,[9] which suggests a possible involvement in heart failure and atherosclerosis. The discovery of U II receptor antagonists would help to elucidate the in vivo pharmacology, as well as afford potential therapeutic agents for the treatment of cardiovascular disorders such as hypertension and heart failure.

Utilizing the techniques of peptide pharmacophore identification pioneered by others,[10] we performed a structure–-function study of U II that established its minimal active size and key structural features. This information, in conjunction with molecular modeling of the GPCR, led to the assembly of a three-dimensional structure of the U-II–receptor complex, which was used to design a more potent U II agonist.

Derivatives of goby U II were screened in a FLIPR-based calcium mobilization assay utilizing the rat receptor expressed in CHO cells[11] as a measurement of agonist activity. Evaluation of close analogs of U II has provided clues to important structural features of the peptide molecule (Table 1). The goby U II peptide was truncated on the N terminus

Table 1. U II agonist activity of cyclic truncated and alanine-scan peptides as measured by calcium flux.

Compound	Sequence[a]	EC_{50} [nM][b]
1 (goby U II)	AGTADCFWKYCV	0.17 ± 0.05[c]
2	GTADCFWKYCV	0.29 ± 0.18
3	TADCFWKYCV	0.11 ± 0.05
4	ADCFWKYCV	0.16 ± 0.05
5	DCFWKYCV	0.10 ± 0.04
6	CFWKYCV	0.76 ± 0.65
7	CFWKYC	1.6 ± 0.9
8	A**A**CFWKYCV	0.60 ± 0.10
9	ADC**A**WKYCV	6.5 ± 1.5
10	ADCF**A**KYCV[d]	> 1000
11	ADCFW**A**YCV[d]	> 1000
12	ADCFWK**A**CV[d]	67 %@1000[e]
13	ADCFWKYCA	0.40 ± 0.20

[a] All peptides were in the cyclic form. The one-letter amino acid code is used. [b] Not corrected for peptide content, unless noted otherwise. [c] Corrected for peptide content. [d] Inactive as an antagonist at 10000 nM. [e] Percent activation at given concentration.

from the 12-mer (**1**) down to the 7-mer (**6**), and finally the valine residue was eliminated from the C terminus to give the 6-mer (**7**). Although a 10-fold reduction in potency is seen in the 6-mer, low-nanomolar agonist activity is retained. An alanine scan was applied to the 9-mer **4** to determine which amino acid residues are most important for U II receptor stimulation. Only replacement of W, K, and Y caused large changes in biological activity, suggesting the importance of the side chains of these amino acids for binding to and activating the U II receptor. Recent structure–function results on human U II are in agreement with our findings.[12]

The significance of the conserved cyclic sequence CFWKYC was confirmed by further truncation (Table 2). Capping of the 6-mer (compound **14**) did not change the

Table 2. Effect of capping, ring removal, and D scan of 6-mer peptide on agonist activity and binding.

Compound	Sequence[a]	EC_{50} [nM][b]	K_i [nM][c]
7	CFWKYC	1.6 ± 0.9[d]	86 ± 13
14	(Ac)-CFWKYC-NH_2	1.6 ± 0.5	13 ± 4
15	(Ac)-SFWKYS-NH_2[e]	> 500	> 10000
16	(Ac)-FWKY-NH_2[e]	57 %@500	> 10000
17	(Ac)-WKY-NH_2[e]	> 500	> 10000
18	(Ac)-C(D-F)WKYC-NH_2[e]	57 %@500	> 10000
19	(Ac)-CF(D-W)KYC-NH_2	26 ± 3	600 ± 30
20	(Ac)-CFW(D-K)YC-NH_2[e]	54 %@500	> 10000
21	(Ac)-CFWK(D-Y)C-NH_2	37 ± 6	1000 ± 100

[a] All cysteine-containing peptides are in the cyclic form. Data corrected for peptide content unless noted otherwise. [b] Agonist activity measured by the calcium flux assay. [c] Binding as measured by displacement of ^{125}I-urotensin II (rat). [d] Not corrected for peptide content. [e] Inactive as an antagonist at 300 nM.

efficacy as was previously reported.[13] However, replacement of the cysteine residues with serine residues to give the acyclic 6-mer **15**, or further removal of amino acids, led to significantly reduced agonist activity (viz. **16** and **17**). Inversion of the configuration of a single amino acid is known to convert an agonist into an antagonist for certain cyclic peptide ligands. An example is the vasoconstrictor endothelin, which acts at a different GPCR.[14] With the U II ligand, reduced agonist potency was seen on stereoinversion of the W and Y residues. However, the modified peptides (**18**–**21**) did not possess U II antagonist properties, as measured by the compounds' ability to block the response induced by U II (**1**).[11] This result demonstrates the importance of the spatial orientation of the WKY pharmacophore for interaction with the receptor. A binding assay,[11] measuring displacement of ^{125}I-labeled rat U II from membranes containing the rat U II receptor, showed activity at higher concentrations (8- to 50-fold) for these short peptides. There appears to be a greater dynamic range in this assay, possibly because of the presence of the native ligand. These smaller peptides might have difficulties in competing with the full-length U II peptide because they have fewer interactions with the receptor, although functionally they can activate the receptor at low concentrations.

The U II receptor is related to the δ-opioid receptor with 27 % identity and 56 % homology.[3] A working model of the latter,[15] which is based on the X-ray structure of rhodopsin,[16] was used as a starting point for our construction of a model of the U II receptor (rat). U II models were placed above the receptor model in various orientations and were then pulled into the receptor with a tether between the lysine nitrogen of U II and the carboxylate of Asp 130 on TM3 of the receptor, a reputed site for such ligand–amine interactions.

It is inherently problematic to establish a plausible structure of a flexible ligand complexed with a second-generation GPCR homology model. However, such a U-II–receptor complex can offer a qualitative assessment of potential interactions and serve to generate hypotheses that are testable with modified ligands. Although a solution-based structure determination using NMR spectroscopy might be more reliable, the resulting structure describes the unbound form of the ligand.[12] The molecular modeling method probes the bound conformation of the ligand, which is more relevant

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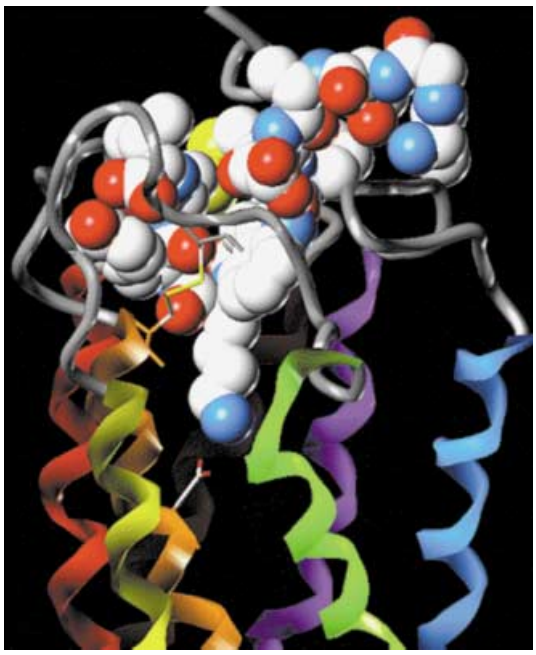

Figure 1. U II (**1**) docked into the U II receptor model.

from a drug design perspective. The docking procedure is based on two central assumptions: the "essential" lysine on the U II ligand binds to Asp 130 on TM3 (similar to many other GPCRs), and interactions between the extracellular loops and the ligand mutually restrict each other's conformations. Thus, as the lysine residue is drawn toward Asp 130, the loops of the receptor and the other parts of the ligand can adjust in a manner that could mimic a native docking process. As a consequence, only a small fraction of the conformational space had to be explored. The receptor models produced from the various starting orientations were visually examined and very few showed well-defined binding sites for the critical W, K, and Y residues of U II (Table 1). A docking model consistent with the molecular recognition of the key side chains of W, K, and Y was selected (Figure 1).

A useful avenue for improving potency and altering efficacy with other GPCR peptide ligands has been the insertion of nonnatural amino acids into the sequence. In Table 3 the capped cyclic 6-mer **14** and analogs modified by point substitution with natural and nonnatural amino acids are compared. Replacement of the Y residue with the related amino acid F maintained potency (**22**) in both the functional and binding assays. Substitution at the same position by A (**23**) drastically reduced potency, as was seen with the 9-mer peptide **12** (Table 1). The combined results with **22** and **23** indicate the primary importance of a hydrophobic group at the Y position of the ligand. The hypothesized tyrosine-binding pocket within the best model of U II (Figure 2) suggested a bulkier side chain for the Y residue, for example, 2-naphthyl.

Introduction of (2-naphthyl)-L-alanine (2-Nal, **25**) actually improved agonist activity slightly in the calcium flux assay relative to the native Y residue, presumably due to enhanced hydrophobic interactions. However, in the binding assay a 100-fold improvement in affinity was noted. Placement of 2-Nal at the W position (**24**) significantly decreased potency in both assays. This loss of activity can be rationalized by the loss of a possible hydrogen bond linking the indole NH group on U II to the receptor. The use of 1-Nal (**26**) led to a potency comparable to that of the parent 6-mer **14**. The D-variant of **25**, **27**, did not exhibit agonist or antagonist activity. Since a large aromatic residue was tolerated at the putative tyrosine-binding site, further elongation of this residue was probed (analogues **28** and **29**). The Bip derivative was similar to the native residue in potency, while the Dpa one was not accommodated, as predicted by the model. The 2-Nal residue was also favorable in the full-length goby U II **1** leading to subnanomolar agonist **30**. Compound **30** was about equipotent to **1** in the functional assay and six-fold more potent in the binding assay ($K_i = 40$ pM), confirming the favorable inter-

Table 3. Effect of the substitution of the tyrosine and tryptophan residues of **14** and **1** with other hydrophobic groups on agonist activity, binding, and vasoconstrictor activity.

Compound	Sequence[a]	EC_{50} [nM][b]	K_i [nM][c]	Response [%][d]
14	(Ac)-CFWKYC-NH_2	1.6 ± 0.5	13 ± 4	11
22	(Ac)-CFWKFC-NH_2	2.1 ± 0.3	18 ± 2	–
23	(Ac)-CFWKAC-NH_2[e]	50 %@1000	> 10000	0
24	(Ac)-CF(2-Nal)KYC-NH_2[f]	31 ± 6	260 ± 50	–
25	(Ac)-CFWK(2-Nal)C-NH_2[f]	0.54 ± 0.2	0.12 ± 0.05	43
26	(Ac)-CFWK(1-Nal)C-NH_2[f]	1.5 ± 0.2	6 ± 1	–
27	(Ac)-CFWK(D-2-Nal)C-NH_2[f,g]	> 1000	1700 ± 200	–
28	(Ac)-CFWK(Bip)C-NH_2[h]	1.8 ± 0.1	6 ± 1	–
29	(Ac)-CFWK(Dpa)C-NH_2[e,i]	> 1000	1600 ± 400	–
1	AGTADCFWKYCV	0.17 ± 0.05	0.26 ± 0.05	91
30	AGTADCFWK(2-Nal)CV[f]	0.34 ± 0.10	0.04 ± 0.02	–

[a–c] See footnotes to Table 2. [d] Vasoconstriction of isolated thoracic aorta at a fixed concentration of 30 nM ($n = 2$). [e] Inactive as an antagonist at 3000 nM. [f] Nal = naphthylalanine. [g] Inactive as an antagonist at 300 nM. [h] Bip = 4-phenyl-L-phenylalanine. [i] Dpa = 4-(2,6-dichlorobenzyloxy)-L-phenylalanine.

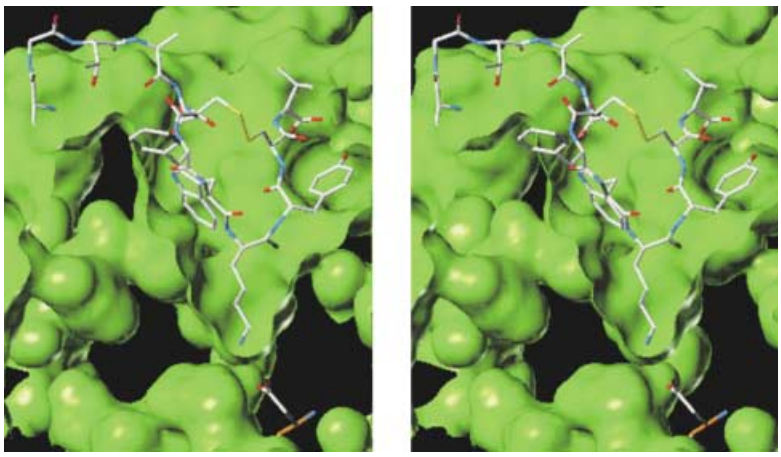

Figure 2. Modeled docking pockets for U II in its receptor. Cut-away view of the U II receptor contact surface.

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action of the 2-Nal group in the tyrosine-binding pocket. The improved potency in the binding assay for analogues **25** and **30** relative to potency in the functional assay is even more striking as compared to other analogues such as **7** and **14**, which had decreased activity in the binding experiment competing with the native ligand. These results point to the beneficial impact of the 2-Nal residue on binding energy.

A selected group of peptides was evaluated for vasoconstrictor activity in a thoracic aorta smooth muscle tissue preparation (Table 3).[11] The rank order of agonist potency at a fixed dose of 30 nM was that predicted by the FLIPR assay results. Goby U II (**1**) was most potent, followed by the 2-Nal 6-mer **25**. Compound **23** was included as a negative control. Again, 2-Nal derivative **25** was more potent than Y-containing analog **14** in this system.

In conclusion, we have conducted a structure–function analysis of the vasoactive peptide U II and employed that information in a docking strategy for the assembly of a ligand–receptor model. This three-dimensional construct has proven useful in rationalizing biological activity and predicting opportunities for potency improvement. In particular, the key W, K, and Y residues of U II, identified as being important for biological function, were found to have significant interactions with the GPCR model. A prominent feature of the receptor model is a tyrosine-binding pocket. As predicted by our working model, the 2-Nal group showed an enhanced interaction with this critical binding region, whereas larger groups were not accommodated. The 2-Nal substituted peptides **25** and **30** showed greater affinity for the receptor than U II as measured by displacement of the radiolabeled native ligand. The interplay between structure–function and molecular modeling studies proved effective as a drug design tool in a case where no tertiary structural information is known. This working model should assist efforts to design nonpeptide ligands as potential U II receptor antagonists.

Experimental Section

Modeling: Starting with a model of the δ-receptor, amino acid residues were mutated according to the homology alignment,[3] and extracellular loops were added with the aid of the Loop–Search routine in Sybyl.[17] The side chains and loops were energy-minimized with AMBER[18] while restraining the backbone of the seven transmembrane (TM) helices. A computational structural analysis of U II (goby) revealed a wide range of low-energy conformations. Two of the lowest-energy structures (found by Monte Carlo conformation searches in a water-solvated model with the MacroModel program[19]) were chosen as starting points and the U II models were placed above the receptor model ($\approx$20 Å tether, see below) in orientations rotated by 30°. The Sander module of AMBER[18] was used to place a tether between the lysine nitrogen of U II and the carboxylate of Asp 130 on TM3 of the receptor. After 5 ps of thermal equilibration at 300 K (a type of simulated annealing), the tether strength (tether energy well parameters: $r_1 = 2$, $r_2 = 2.5$, $r_3 = 3.5$, $r_4 = 4.0$, $r_{k2} = 32$, $r_{k3} = 128$) was increased (0 to 32 kcal mol^{-1} Å^2) over 3 ps, held at the top value for 1 ps, and then turned off for 10 ps. During this process, only the backbones of the helices were kept stationary; the ligand, extracellular loops, and side chains were free to move about and interact. The resulting complexes were selected on the basis of the structure–activity relationships available at that time (Table 1). The receptor model with the best-defined pockets for the tryptophan, lysine and tyrosine residues of the ligand was chosen as working model.

Peptides:[20] Goby U II (**1**) was purchased from Bachem Bioscience Inc., King of Prussia, PA, USA (no. H-6950). Peptides **2**–**30** were prepared from the C terminus to the N terminus by the Merrifield method on a solid resin support.[21] Depending on whether the C terminus was desired as an amide or acid, the resin was selected from an Fmoc-amide resin or Wang resin, respectively. During the synthesis, lysine (Boc), cysteine (Trt), aspartic acid (*t*Bu), serine (*t*Bu), threonine (*t*Bu), and tyrosine (*t*Bu) all contained side chain protecting groups (given in brackets). N-acylation was done as the last step of the synthesis by using acetic anhydride. Cleavage of the peptide from the resin was achieved with 95% trifluoroacetic acid, which also removed the protecting groups. The peptide was purified prior to cyclization by reversed-phase HPLC and then cyclized directly with potassium ferricyanide in aqueous ammonium bicarbonate. Since side products, for example dimeric products, were often formed during this cyclization, the post-cyclization product was chromatographed to isolate the desired peptide. All the peptides were analyzed by electrospray mass spectrometry (Finnegan LCQ instrument) and analytical HPLC. All compounds exhibited the correct mass and most were at least 90% pure (HPLC). Peptides **14**–**30** were assayed for peptide content by amino acid analysis.[11] The biological data were calculated based on the amount of pure peptide present in the sample. All unique structures (disregarding stereoisomers) within compounds **14**–**30** were analyzed for accurate mass by high-resolution fast-atom bombardment mass spectrometry with a Micromass Autospec E double-focusing mass spectrometer (polyethylene glycol as internal mass reference); only the D-peptides **18**–**21** were not analyzed by this method.[11]

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[20] Abbreviations: Fmoc = 9-fluorenylmethoxycarbonyl, Boc = *tert*-butoxycarbonyl, Trt = trityl.
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A Cluster Rearrangement of an Open Cubane (Cu_4Br_4) to a Prismane (Cu_6Br_6) in a Copper(I)–Olefin Network**

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The rational design and self-assembly of copper(I)–olefin coordination polymers possessing high thermal stability has been the focus of intense interest in recent times.[1] While these materials possess many of the general features normally associated with coordination polymers, the inclusion of bridging ligands that are capable of π bonding offers the possibility of unusual and novel properties. The Cu^I–olefin complexes examined so far have demonstrated an ability to act as fluorescent sensors,[1] and have potential applications in areas such as olefin separation[2] and enantioseparation.[3]

To the best of our knowledge, the presence of clusters, such as cubanes, within Cu–olefin coordination polymers is unknown, however there are a number of metal–organic frameworks with clusters acting as connecting units. Such materials have demonstrated gas-storage capabilities as well as exhibiting magnetic and catalytic properties.[4–6] The successful generation of networks incorporating olefin coordination to a copper cluster represents an exciting challenge in modern supramolecular and organometallic chemistry. With this in mind, we have studied the reactions of triallyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione (TTT) with CuBr at different temperatures. Herein we report the synthesis, solid-state structures, and some electrochemical properties of two materials generated from such reactions.

The reaction between TTT and CuBr in methanol at 50–60 °C in a sealed tube yielded a product with the formula $[Cu_4Br_4(TTT)_2]_n$ (**1**), which was examined by single-crystal X-ray diffraction.[7] In this complex, Cu_4Br_4 clusters are linked by TTT ligands to form a polymeric chain (Figure 1). The Cu and Br atoms occupy corners of a distorted cube, however as is apparent in Figure 1, there are only 10 Cu–Br bonds in each cluster resulting in an open-cubane arrangement (a closed-cubane arrangement would have a bond along each edge). The open-cubane unit has two unique Cu^I atoms each being coordinated by an olefin moiety and two bromide ions in an approximate plane with the metal center. One of the unique Cu atoms is coordinated to an extra bromide ion located within the cubane unit. This third Cu–Br bond is almost perpendicular to the plane of the other donor atoms, and with a length of 2.95 Å it is considerably longer than the other Cu–Br bonds.

Figure 1. The chain structure of **1** which extends in the *c* direction (Cu green, Br pink, O red, N blue, C black). The open-cubane unit is highlighted with yellow bonds. H atoms have been omitted for clarity. Typical bond lengths [Å]: Cu–Br 2.422–2.395, Cu–C 2.063–2.106, coordinated olefin C=C 1.347–1.402.

Only two of the three arms of the TTT ligand are involved in coordination to the cubane units. The terminal carbon atom of the third arm is disordered over two sites. As is apparent in Figure 1, small cavities within the chain exist with approximate dimensions of 5 × 7.5 Å. These are formed by double-ligand bridges linking neighboring cubane units (a pseudo-3D network representation of **1** can be seen in the Supporting Information).

There are many examples of cubane clusters serving as the connecting node[8] in metal–organic coordination polymers, however, to the best of our knowledge, **1** is only the second example of olefin-based ligands linking cubane-type units in coordination polymers.[8e]

The complex $[Cu_6Br_6(TTT)_2]_n$ (**2**), was formed in the reaction between CuBr and TTT in ethanol at approximately 90 °C. The solid-state structure of **2**[7] (Figure 2a) indicates the

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