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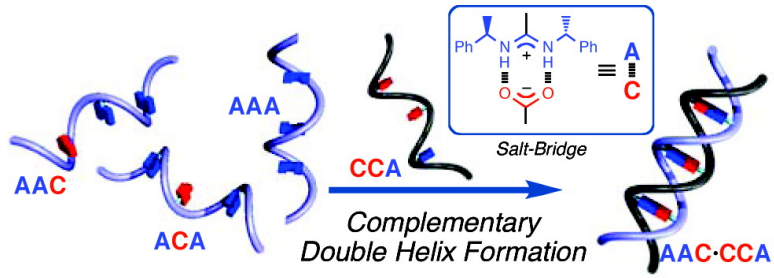


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# Sequence- and Chain-Length-Specific Complementary Double-Helix Formation

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***Abstract:*** The artificial sequential strands consisting of two, three, or four *m*-terphenyl groups joined by diacetylene linkers with complementary binding sites, either the chiral amidine (A) or achiral carboxyl (C) group, were synthesized in a stepwise manner. Using circular dichroism and $^1$H NMR spectroscopies along with liquid chromatography, we showed that, when three dimeric molecular strands (AA, CC, and AC) or six trimeric molecular strands (AAA, CCC, AAC, CCA, ACA, and CAC) were mixed in solution, the complementary strands were sequence-specifically hybridized to form one-handed double-helical dimers AA·CC and $(AC)_2$ or trimers AAA·CCC, AAC·CCA, and ACA·CAC, respectively, through complementary amidinium−carboxylate salt bridges. Upon the addition of CCA to a mixture of AAA, AAC, and ACA, the AAC·CCA double helix was selectively formed and then isolated from the mixture by chromatography. Moreover, the homo-oligomer mixtures of amidine or carboxylic acid from the monomers to tetramers (A, AA, AAAA, C, CC, and CCCC) assembled with a precise chain length specificity to form A·C, AA·CC, and AAAA·CCCC, which were separated by chromatography.

## Introduction

The double helix of DNA is exquisite from the standpoint of structures and functions that rely on its one-handedness and complementarity of the strands, motivating chemists to develop synthetic helical polymers and oligomers.[1−9] While a number of synthetic strands that fold into a single helix have been reported,[1,2] only a few structural motifs are available for double helices other than the DNA analogues.[2−9] Most of these, however, lack the important feature of DNA, the complementarity of the strands. The sequence-specific duplex formation of DNA is the most essential process for self-replication and protein synthesis. The first step toward breaking the monopoly of nature may be the construction of double helices with a

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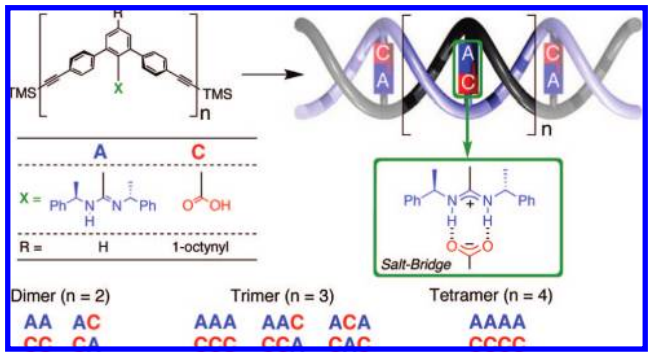


***Figure 1.*** Structures of *m*-terphenyl-based molecular strands bearing amidine and/or carboxyl groups and an illustration of double-helical oligomers consisting of complementary molecular strands stabilized by amidinium−carboxylate salt bridges. "A" and "C" denote the monomer units bearing the chiral amidine and achiral carboxyl groups, respectively.

controlled helicity consisting of complementary and sequential strands. Taking advantage of the high stability and well-defined directionality of the amidinium−carboxylate salt bridges,[10] we have recently designed and synthesized a heterostranded double helix with a controlled helix sense that consists of an optically active dimeric amidine and a complementary achiral carboxylic acid strand with *m*-terphenyl backbones, the duplexation of which relies on salt bridge formation (Figure 1).[8] The amidinium−carboxylate salt bridges have also been widely employed to construct a number of supramolecular diads[10a−f] and capsules[10g] and utilized as supramolecular junctions for crystal engineering,[10h] molecular imprinting,[10i] and autocatalytic systems,[10j] owing to the high association constants and well-defined geometry of the charge-assisted, double hydrogen bonding and the high tolerance toward various functional groups.

We now report the sequence-specific binding of complementary molecular strands with sequential information along with the chain length discrimination of homo-oligoamidines and -oligocarboxylic acids through specific double-helix formation. In seeking to understand the principles that underlie the precise discrimination behavior of biological molecules such as DNA, chemists have developed a variety of molecular and supramolecular systems endowed with a self-sorting ability.[11−15] In most cases, their self-sorting has been achieved by the formation of (supramolecular) macrocyclic species,[11] while quite a few systems perform self-sorting by reading information on the linear molecular strands, such as the chain lengths and sequences, through formation of the multiple-stranded complexes.[12−15] Double helices could be taken as a versatile platform having an advantage in the recognition of the sequence and chain length between the strands,[12,13] as exemplified by the seminal examples of the self-sorting of double-stranded Cu(I) helicates,[12a] since the geometrical feature that the two strands are intertwined with each other in close proximity is likely to work in favor of the precise recognition of the structures of the molecular strands. Our double-helical oligomers are capable of discriminating structural information on the molecular strands, such as chain lengths and sequences through double-helix formation driven by the complementary amidinium−carboxylate salt bridge that are reminiscent of the complementary nucleic acid base pairs in DNA. Furthermore, the thermodynamically stable salt bridges enable the separation of the self-sorting mixture by chromatography, as opposed to other dynamic complexes that require the aid of covalent bonds, such as the disulfide bond, for separation and isolation.[14a,15a]

## Results and Discussion

**Synthesis of Oligomeric Strands.** Molecular strands consisting of two, three, or four *m*-terphenyl groups joined by diacetylene linkers with complementary binding sites, either the chiral amidine (A) or achiral carboxyl (C) group, were synthesized in a stepwise manner according to Schemes 1−3. In our previous study,[8a] we synthesized the complementary dimer strands without side chains (AA·C′C′, C′ = C (R = H) in Figure 1) and investigated their double-helix formation. However, an increase in the chain lengths of the double helices resulted in a drastic solubility decrease in most common solvents, which was improved by introducing a side chain onto the "tail" of the carboxylic acid units.[8f] We employed the carboxylic acid unit with a 1-octynyl chain as a solubility enhancer throughout this study. In addition to the solubility, the use of less polar solvents with a low viscosity such as $CDCl_3$ gives rise to $^1$H NMR spectra with high resolution, compared to those measured in

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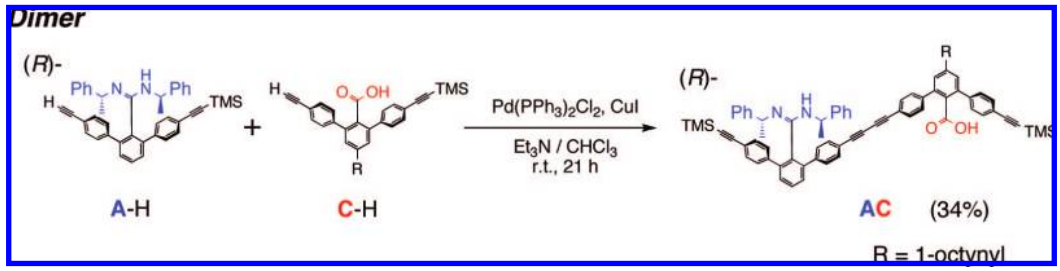
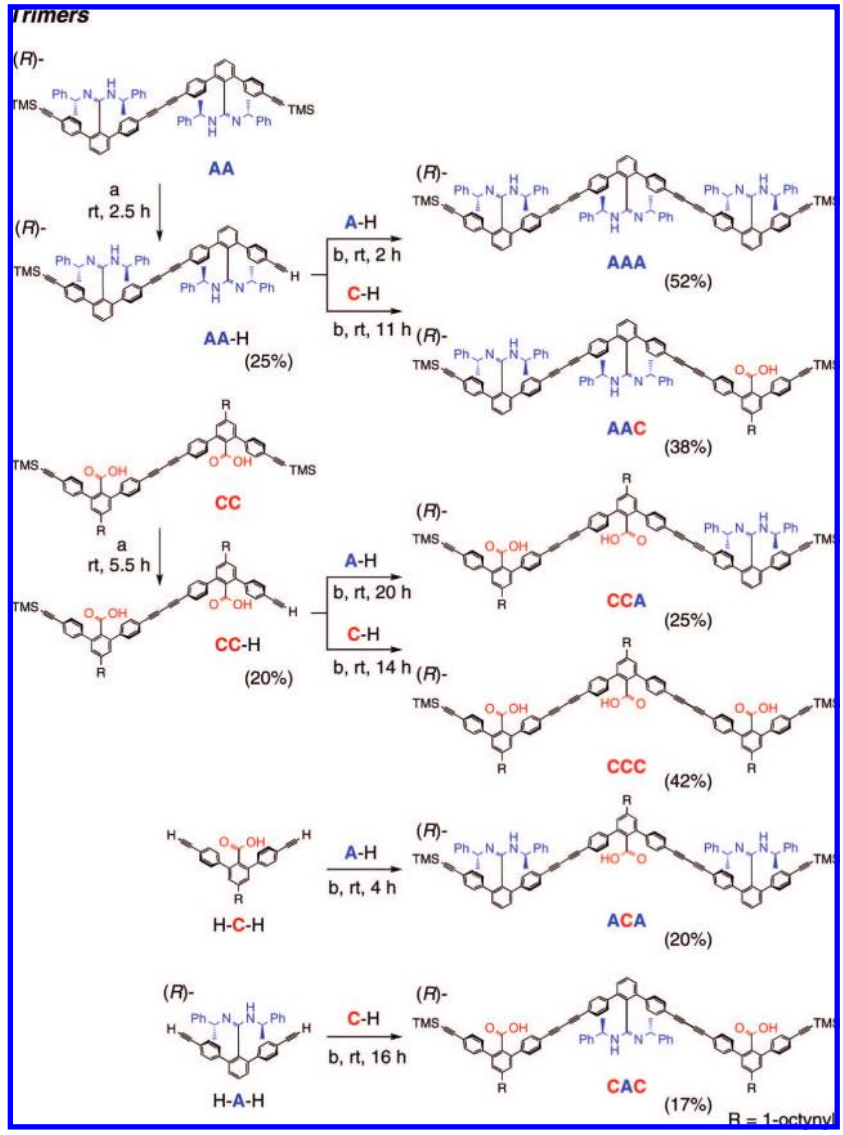
***Scheme 1.*** Synthesis of the Self-Complementary Dimer AC




***Scheme 2.*** Synthesis of Trimers AAA, AAC, CCA, CCC, ACA, and CAC[a]




[a] Reagents and conditions: (a) TBAF/THF; (b) $PdCl_2(PPh_3)_2$, CuI, $Et_3N-CHCl_3$.

polar solvents such as DMSO-$d_6$ and $D_2O$. The self-complementary novel dimeric strand consisting of a chiral amidine and an achiral carboxyl unit, AC, was synthesized by Glaser-type coupling[16] of the monosilylamidine, A−H,[8a] and the monosilylated carboxylic acid, C−H,[8f] using a palladium catalyst (Scheme 1). The reaction, the progress of which was monitored by TLC, was terminated after both A−H and C−H had been completely consumed. AC was obtained in 34% yield along with the homocoupling side products of AA and CC. The yield of AC is moderate, considering that the reaction is nonselective, giving the cross-coupling product with a theoretical maximum yield of 50%. The six trimeric strands consisting of *m*-terphenyl groups joined by diacetylene linkers with complementary binding sites, either the chiral amidine or achiral carboxyl group, namely, AAA, CCC, AAC, CCA, ACA, and CAC, were synthesized in a stepwise manner (Scheme 2). The monosilylated amidine dimer, AA−H, was obtained from AA[8a] by treatment with tetrabutylammonium fluoride (TBAF) in THF. AAA and AAC were prepared by Glaser-type coupling between AA−H and A−H or C−H, respectively. Similarly, the carboxylic acid

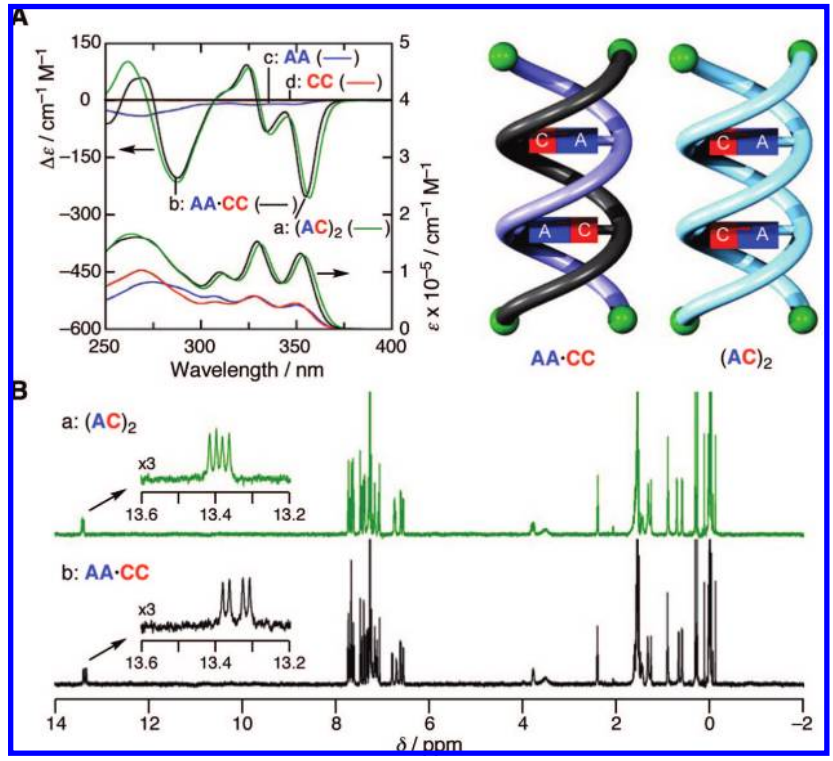
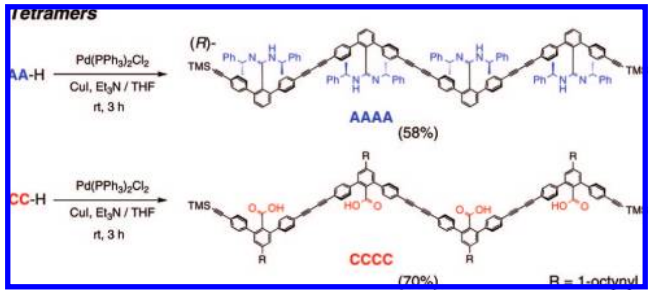



***Figure 2.*** (A) CD and absorption spectra of $(AC)_2$ (a, green), AA·CC (b, black), AA (c, blue), and CC (d, red) in $CDCl_3$ (0.10 mM, 25 °C, cell length 0.1 cm). (B) $^1H$ NMR spectra of $(AC)_2$ (a, green) and AA·CC (b, black) in $CDCl_3$ (0.10 mM, 25 °C).

***Scheme 3.*** Synthesis of Tetramers AAAA and CCCC




dimer bearing two 1-octynyl chains, CC, was treated with TBAF to afford the monosilylated acid dimer, CC−H, which was further allowed to react with A−H and C−H under Glaser-type coupling conditions to yield CCA and CCC, respectively. The trimeric strands with an alternate sequence, ACA and CAC, were also synthesized by Glaser-type coupling reactions between H−C−H and A−H, and H−A−H and C−H, respectively. The six trimeric strands were obtained in moderate yields (17−52%), due to the nonselective nature of the coupling reaction. The homotetramers of amidine and carboxylic acid, AAAA and CCCC, were obtained from AA−H and CC−H, respectively, in good yields by Glaser-type homocoupling (Scheme 3). All the oligomers were purified by column chromatography and characterized and identified using $^1H$ and $^{13}C$ NMR spectroscopies, elemental analyses, and mass measurements (see the Supporting Information (SI)).

**Complementary Double-Helix Formation.** AC formed a self-complementary homostranded double helix, $(AC)_2$, through salt bridge formation in $CDCl_3$, as confirmed by $^1H$ NMR, circular dichroism (CD), and electron-spray ionization mass (ESI-MS) spectroscopies (Figure 2 and the SI). The CD spectrum of $(AC)_2$ exhibited intense Cotton effects in the absorption region of 250−370 nm, suggesting a helix sense bias induction, as reported for the analogous complementary heterostranded double-helix dimer AA·C′C′ without the 1-octynyl groups.[8a] $(AC)_2$ appears to have a right-handed double helix on the basis of the X-ray structure of the heterostranded double helix AA·C′C′, although the CD spectra of the two double helices $(AC)_2$ and AA·CC have spectral patterns slightly different from each other, especially at around 270 nm, reflecting the difference in their sequences.

The complementary homotrimers AAA and CCC formed the corresponding double helix AAA·CCC in $CDCl_3$ through salt bridge formation. The other complementary heterotrimers, AAC and CCA, and ACA and CAC, also formed the double helices AAC·CCA and ACA·CAC, respectively, with the aid of salt bridge formation. Similarly to the dimeric double helices, intense CD signals were observed for the trimeric double helices in the absorption region of 250−370 nm, indicating that they adopted a preferred right-handed double helix with an excess one-handedness (Figure 3A−C). The CD spectra of the three double helices also have spectral patterns slightly different from each other, especially in the region of 250−300 nm, due to the difference in their sequences.

The complementary homotetramers AAAA and CCCC also assembled into the double helix AAAA·CCCC through the formation of four salt bridges. Similarly to the cases of the homodimers and -trimers, AAAA·CCCC showed intense Cotton effects in the region above 250 nm, which indicated excess right-handed double-helix formation (Figure 3D).

**Sequence-Specific Double-Helix Formation.** When the three dimeric strands AA, CC, and AC were mixed together in $CDCl_3$, the CD and $^1H$ NMR spectra immediately changed

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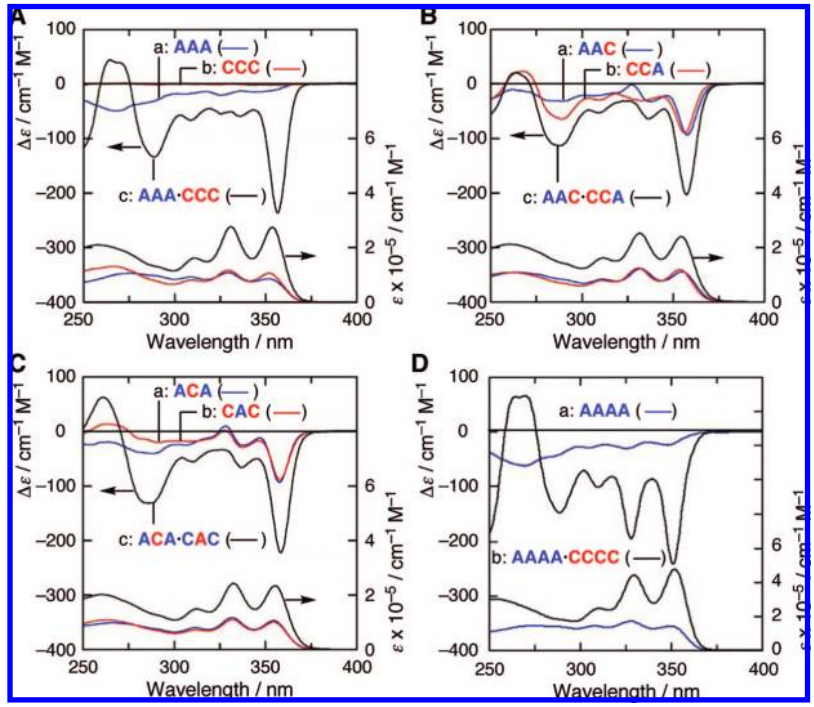



***Figure 3.*** CD and absorption spectra of AAA (a, blue), CCC (b, red), and AAA·CCC (c, black) (A), AAC (a, blue), CCA (b, red), and AAC·CCA (c, black) (B), ACA (a, blue), CAC (b, red), and ACA·CAC (c, black) (C), and AAAA (a, blue) and AAAA·CCCC (b, black) (D) in $CDCl_3$ (50 $\mu$M, cell length 0.1 cm) at 25 °C.

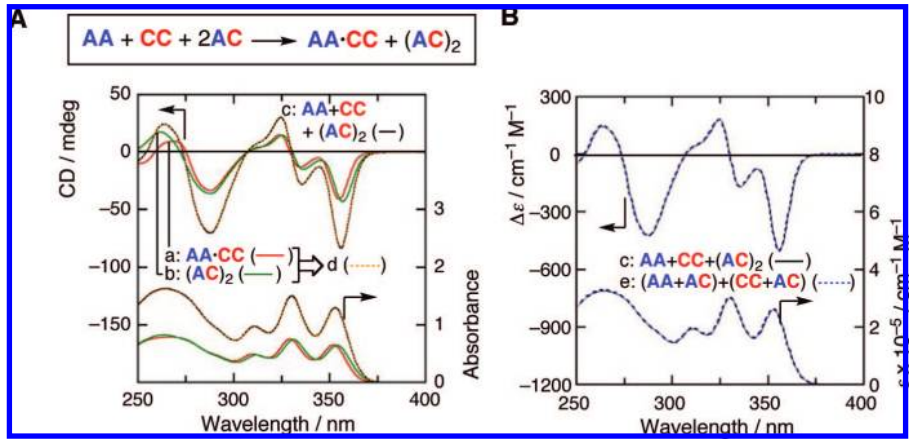



***Figure 4.*** (A) CD and absorption spectra of AA·CC (a, red), $(AC)_2$ (b, green), and a mixture of AA, CC, and $(AC)_2$ (c, black, after mixing in this order) in $CDCl_3$ at 25 °C (50 $\mu$M for each, cell length 0.1 cm), and the simulated CD and absorption spectra for a mixture of separately prepared double-helical dimers (d, dashed orange). (B) CD and absorption spectra of a mixture of AA, CC, and $(AC)_2$ (c, black, after mixing in this order) and an equimolar mixture of AA + AC and CC + AC (e, dashed blue, after mixing in this order) in $CDCl_3$ at 25 °C (50 $\mu$M for each, cell length 0.1 cm).

and became precisely identical to the simulated spectra obtained by the summation of each double helix AA·CC and $(AC)_2$ (Figures 4A and S3 (SI)), indicating that the molecular strands sequence-specifically hybridized to form the corresponding one-handed double helices through the complementary amidinium−carboxylate salt bridges. The CD and $^1$H NMR spectra of the mixture did not change at all regardless of the orders of addition of the dimeric strands (Figures 4B and S3 (SI)). For comparison, we mixed an equimolar amount of AA and AC, which are not complementary to each other, in $CDCl_3$, forming the self-complementary double helix $(AC)_2$ together with AA remaining as a single strand without any other chemical species, as evidenced by the identical experimental and simulated CD and $^1$H NMR spectra (Scheme 4A and Figure S1 (SI)). The combination of CC and AC resulted in the formation of a mixture of $(AC)_2$ and CC·AC along with the free CC, as suggested by the CD and $^1$H NMR spectra, which are different from the simulated spectra for the mixture of $(AC)_2$ and the free CC (Scheme 4B and Figures S2A and S2B (SI)). Since the chain exchange between $(AC)_2$ and CC·AC is slow on the $^1$H NMR time scale, they gave separated signals, from which the ratio of $(AC)_2$ to CC·AC was estimated to be 1:1 (Figure S2C (SI)). The formation of CC·AC was presumably promoted by the dimerization of the carboxylic groups, though it is much smaller ($\sim 10^2$ $M^{-1}$ in $CHCl_3$)[17] than that of the amidinium−carboxylate salt bridges ($>10^6$ $M^{-1}$ in $CDCl_3$).[10i]

(17) I'haya, Y.; Shibuya, T.-i. *Bull. Chem. Soc. Jpn.* **1965**, *38*, 1144–1147.

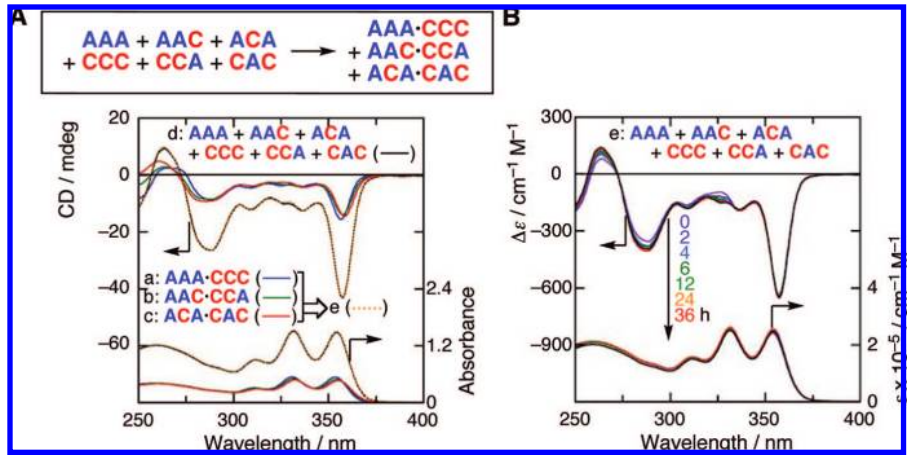
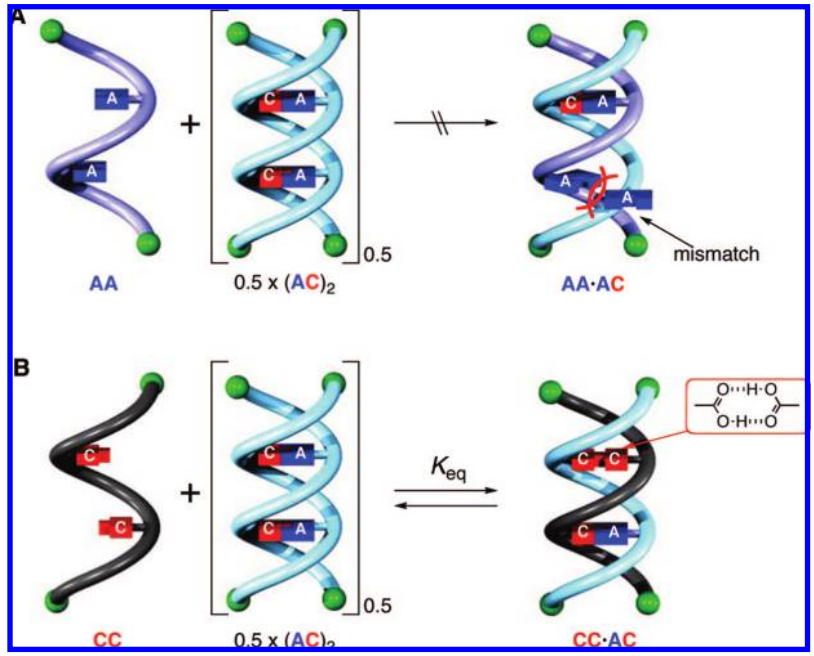



***Figure 5.*** (A) CD and absorption spectra of AAA·CCC (a, blue), AAC·CCA (b, green), ACA·CAC (c, red), and an equimolar mixture of AAA, AAC, ACA, CCC, CCA, and CAC (d, black, after mixing in this order; the sample had been allowed to stand for 36 h) in $CDCl_3$ ($2 \times 10^{-6}$ M, 25 °C) and the simulated CD and absorption spectra for an equimolar mixture of separately prepared double-helical trimers AAA·CCC, AAC·CCA, and ACA·CAC (e, dashed orange) in $CDCl_3$ at 25 °C (2.0 $\mu$M for each, cell length 1 cm). (B) Time course of CD and absorption spectral changes of an equimolar mixture of AAA, AAC, ACA, CCC, CCA, and CAC in $CDCl_3$ (2.0 $\mu$M for each, 25 °C) after mixing in this order.

***Scheme 4.*** Schematic Illustrations of the Duplex Formation between the Dimeric Strands That Are Not Complementary to Each Other




When equimolar amounts of the six trimers AAA, CCC, AAC, CCA, ACA, and CAC were mixed together in $CDCl_3$, the CD spectrum gradually changed with time (Figure 5B). After 36 h, the mixture reached equilibrium, and the resultant CD spectrum became precisely identical to the simulated CD spectrum obtained by the summation of each double helix AAA·CCC, AAC·CCA, and ACA·CAC (Figure 5A), indicating that the six molecular strands sequence-specifically hybridized to form the corresponding double helices through the complementary amidinium−carboxylate salt bridges. As in the case of the dimeric double helices, the addition order of the strands did not affect the sequence-specific sorting of the trimeric strands during double-helix formation. The $^1$H NMR spectrum of an equimolar mixture of the six trimeric strands also supports the perfect sorting of the six trimeric strands (Figure S5 (SI)); the $^1$H NMR spectrum was identical to the simulated spectrum obtained by the summation of those of the separately prepared, three double helices with a complementary sequence. It should be mentioned here that some trimeric strands with "partially" self-complementary sequences, such as AAC, ACA, CAC, and CCA, formed a partial double helix in a staggered fashion through the salt bridges, showing weak, but distinct Cotton

(18) Some trimer strands also formed double helices with partially complementary sequences (AAA and CAC, AAA and CCA, AAC and ACA, AAC and CAC, AAC and CCC, ACA and CCA, ACA and CCC, and CAC and CCA), exhibiting apparent Cotton effects. However, the CD intensities, CD patterns, and/or stabilities of the double helices obtained by dilution CD measurements are different from those of the fully complementary pairs. The duplex formation between the partially complementary strands is too complicated to be fully understood, because of the presence of the concentration dependence as well as the self-dimerization, whereas the double helices of the fully complementary pairs are highly stable and did not exhibit either concentration dependence or self-dimerization over a range of 1−50 $\mu$M.

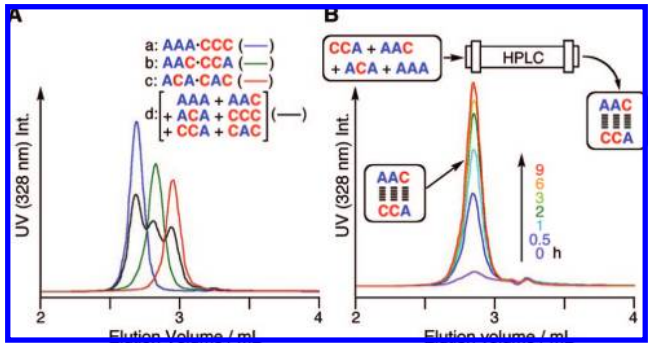



***Figure 6.*** (A) UV (328 nm) detected HPLC chromatograms of AAA·CCC (a, blue, 29 $\mu$M, 7.0 $\mu$L), AAC·CCA (b, green, 33 $\mu$M, 6.0 $\mu$L), ACA·CAC (c, red, 27 $\mu$M, 7.3 $\mu$L), and an equimolar mixture of six strands, AAA, AAC, ACA, CCC, CCA, and CAC (d, black, 10 $\mu$M for each, 10 $\mu$L, after mixing in this order; the sample had been allowed to stand for 36 h). (B) Sequence-selective double-helix formation and its isolation: time-dependent changes in the HPLC chromatogram of an equimolar mixture of four trimers, AAA, AAC, ACA, and CCA (CCA was added 12 h after AAA, AAC, and ACA were mixed in $CHCl_3$), 0−9 h after mixing in $CHCl_3$ at 25 °C. HPLC conditions: column, TSKgel Silica-60 (Tosoh, 0.46 (∅) × 25 cm); eluent, $CHCl_3$/hexane (1:1, v/v); flow rate, 1.0 mL/min; column temperature, 30 °C.

effects with CD patterns different from those of the double helices with the "perfectly" complementary sequences (Figure S4 (SI)).[18]

This sorting by sequence was further evidenced by high-performance liquid chromatography (HPLC). The mixture of the six strands was then subjected to HPLC using a silica gel column with $CHCl_3$/hexane (1:1, v/v) as the eluent (Figure 6A). The three double helices AAA·CCC, AAC·CCA, and ACA·CAC were sequence-specifically eluted without dissociation by virtue of the strong salt bridges, being separated and eluted in this order as supported by the identical elution times of each separately prepared double helix. Under this normal-phase condition, each trimer itself was strongly adsorbed on the column and could not be eluted at all. This drastic change in the elution behavior is attributed to the salt bridges, resulting in masking the highly polar amidine and carboxyl groups with the less polar *m*-terphenyl backbones via duplex formation.

When an equimolar mixture of the three trimeric strands AAA, AAC, and ACA was injected into the HPLC instrument, no strand was eluted under the same normal-phase condition ($CHCl_3$/hexane (1:1, v/v)) due to no complementary pairing. However, a single peak emerged, and its intensity increased with time upon the addition of CCA to the mixture, which is the strand complementary to AAC (Figures 6B and S6 (SI)). The peak was identified as the double helix AAC·CCA by its retention time and CD spectrum (Figure S10 (SI)). The other two strands, AAA and ACA, adsorbed on the stationary phase under the stated conditions, were eluted after the eluent was changed to that containing 30 vol % THF (Figure S9 (SI)). Thus, the AAC strand was successfully extracted from the mixed strands by its complementary strand, CCA, due to the sequence-specific binding through salt bridge formation. Similarly, the CCC sequence-selectively bound its complementary strand, AAA, in a mixture of AAA, CAC, CCA, and CCC, and ACA complexed selectively with CAC in a mixture of ACA, CAC, CCA, and CCC (Figures S7 and S8 (SI)).

**Chain-Length-Specific Double-Helix Formation.** The chain length, or the number of repeating units, is another kind of intrinsic information about oligomeric molecular strands. As with the sequence discrimination, it is not a very easy task to discriminate chain lengths, and only a few examples have been reported to date.[12,13] Therefore, it is of great interest to investigate the chain-length discrimination ability of our salt-bridge-based double-helical oligomers. Upon mixing A, C, AA, CC, AAAA, and CCCC in $CDCl_3$, the absorption, CD, and $^1$H NMR spectra quickly became exactly the same as those simulated for a mixture of A·C, AA·CC, and AAAA·CCCC, irrespective of the addition order (Figures 7 and S11 (SI)), indicating that the oligomers formed double helices, with their complementary strands having the same chain lengths. Furthermore, when the mixture was subjected to SEC using polystyrene gel columns with $CHCl_3$ as the eluent, the mixture was eluted without dissolution as in the case of the complementary trimers, because of the stable salt bridges, and the SEC chromatogram of the mixture showed three separated peaks that were assigned to AAAA·CCCC, AA·CC, and A·C from those with the shorter retention times (Figure 8).

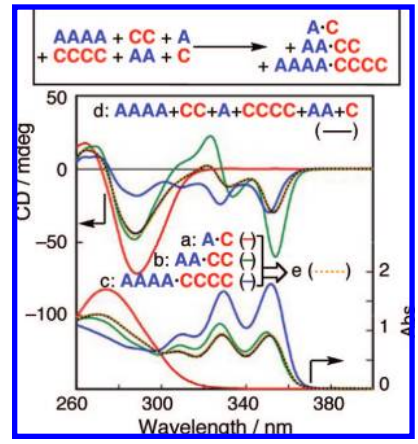



***Figure 7.*** CD and absorption spectra of A·C (a, red, 150 $\mu$M), AA·CC (b, green, 75 $\mu$M), AAAA·CCCC (c, blue, 37.5 $\mu$M), and a mixture of AAAA (12.5 $\mu$M), CC (25 $\mu$M), A (50 $\mu$M), CCCC (12.5 $\mu$M), AA (25 $\mu$M), and C (50 $\mu$M) (d, black, after mixing in this order) in $CDCl_3$ at 25 °C (cell length 1 cm) and the CD and absorption spectra simulated for a mixture of separately prepared double helices A·C (50 $\mu$M), AA·CC (25 $\mu$M), and AAAA·CCCC (12.5 $\mu$M), (e, dashed orange).

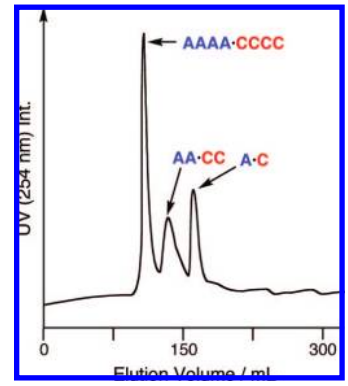



***Figure 8.*** SEC chromatogram of a mixture of AAAA (12.5 $\mu$M), CC (25 $\mu$M), A (50 $\mu$M), CCCC (12.5 $\mu$M), AA (25 $\mu$M), and C (50 $\mu$M) in $CDCl_3$ at 25 °C (after mixing in this order) (column, JAIGEL-1H and -2H (JAI, 1 (∅) × 60 cm); eluent, $CHCl_3$; flow rate, 3.8 mL/min).

## Conclusions

We have synthesized a series of dimeric, trimeric, and tetrameric strands consisting of *m*-terphenyl backbones linked by diacetylene linkers with chiral amidine and/or achiral carboxylic acid units as the complementary binding sites. The

trimeric strands with various sequences sequence-specifically formed double helices with the molecular strands bearing their complementary sequences. A mixture of homo-oligomers of amidines and carboxylic acids spontaneously yielded double helices consisting of the complementary strands with the same lengths. Thus, our salt-bridge-based double helices have the capability of precisely discriminating the sequences and chain lengths of molecular strands through complementary double-helix formation. In addition, the highly stable salt bridges enable the separation and isolation of the double helices without dissolution by chromatography, contrary to the other supramolecular self-sorting systems that may not be separable without the aid of covalent bonds. Our results provide the first artificial designer molecules having a dynamic self-sorting ability with respect to sequences and chain lengths that are separable due to the strong salt bridges, which may lead to the first step toward a totally artificial self-replication system.

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**Supporting Information Available:** Full experimental details of the double-helix formation of the dimeric, trimeric, and tetrameric strands. This material is available free of charge via the Internet at http://pubs.acs.org.

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