Large-scale and chromatography-free synthesis of an octameric quinoline-based aromatic amide helical foldamer

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The synthesis of helical aromatic oligoamide foldamers derived from 8-amino-2-quinolinecarboxylic acid is described. The precursors are commercially available and products up to and including the octamer are obtained. The procedure covers the synthesis of the monomer, reduction of N-terminal nitro groups into amines, saponification of C-terminal methyl esters to form carboxylic acids, and coupling of amines and acids to form amides via acid chloride activation. Emphasis is given to how these reactions can be scaled up and how purification can be greatly simplified using recrystallization methods, thus providing considerable improvements over previously described procedures. As an illustration of the improvement, 8.4 g of an octamer can now reliably be prepared from a nitro-ester monomer in a matter of 8 (working) weeks without any chromatography.

INTRODUCTION

Aromatic oligoamide foldamers are synthetic oligomers derived from aromatic amino acids that adopt stable folded conformations in solution[1–4]. Recent developments in this field suggest that the range of 3D structures covered by these folded molecules may be as rich as that of their aliphatic counterparts, namely α-peptides and their homologs. For example, helices[5–7] and macrocycles[8,9] of narrow or wide diameters; double[10], triple[11] and quadruple helices[12]; linear strands[13] and sheets[14,15]; and tertiary-like objects[16–19] involving several helical segments have all been produced using aromatic amide backbones. In addition, some folding patterns unknown in peptides have been generated, such as helices whose diameter varies along the sequence[20,21] or unconventional ladder-like helices[22]. This rapid progress has put aromatic amide foldamers in the spotlight, and the field is currently attracting much interest. In particular, a growing number of reports demonstrate their potential for biological applications. Their aromatic nature makes them resistant to proteolytic degradation, and their robust and predictable conformations provide efficient scaffolds to project proteinogenic side chains at a defined position in space. Thus, amphipathic aryl amide oligomers have been fine-tuned to show selective antimicrobial activity[23] or to interfere with heparin function[24]. Linear aryl amides have been used as α-helix mimetics designed to disrupt protein-protein interactions[25,26], and helical oligomers bearing cationic side chains feature cell-penetrating properties[27,28] and selective binding to G-quadruplex DNA[29,30].

This article focuses on the large-scale synthesis of helically folded oligoamides, up to the octamer, of 8-amino-4-isobutoxy-2-quinolinecarboxylic acid (**Fig. 1**), one of the most popular families of aromatic amide foldamers. The procedures below will find applicability in three different contexts. First, the very compounds described here possess the rare advantages of being large (up to 2 kDa), structurally very well defined and easily accessible on a large scale. As such, they represent useful building blocks to prepare large biomimetic architectures such as protein-sized folded structures[17,19], long synthetic helices having up to 40 units[31] or, as was shown recently, large helical containers that can encapsulate a guest molecule[21]. Indeed, the field is now at a stage where what was an end product in 2003 (i.e., the octamer[6] described in this article) is now used as a starting material to prepare artificial tertiary structures. The longest sequence prepared in our laboratory on the basis of the octamer described here comprises 64 units, which amounts to over 25 helix turns and an overall length of 9 nm (T.Q., K. Srinivas, V. Maurizot, I.H., unpublished data). Its synthesis will be reported in due course. Second, the same procedures serve, after some adjustments, to produce variants having other side chains than isobutoxy groups, including water-solubilizing side chains (see Experimental design). For example, a variant of the helical octamer bearing ornithine-like ammonium side chains shows promise for several biological applications[27–30], and a variant bearing oligoethylene oxide side chains was recently prepared to be endowed with solubility in a broad range of media[32]. Anionic, aspartate-like side chains have been introduced as well[30,33]. Third, these procedures can be relevant in multiple ways to many of the aromatic amide foldamers referred to above. Even though some specific problems may occur in any given foldamer family[34], and thus some specific solutions may be needed (see Experimental design), a number of the problems resolved here are likely to be commonly encountered upon optimizing and scaling up other protocols.

Because of the aromatic nature of the backbone, synthetic procedures to prepare aromatic amide foldamers differ markedly from those of aliphatic peptides. Aromatic amines are poor nucleophiles, especially when they are carried by electron-poor aromatics. Their coupling is efficient with strongly activated acids, typically acid chlorides. An important feature of aromatic amide foldamer conformations is their very high stability in a wide range of solvents, including those in which synthetic procedures are carried out. This is particularly acute for the oligomers discussed in this paper whose stability reaches record levels[32,35]. Folding generates steric hindrance, which may slow down reactions and potentially result in lower yields. In response, some strategies have been proposed to transiently disrupt folding so as to facilitate synthesis via the conversion of secondary amides

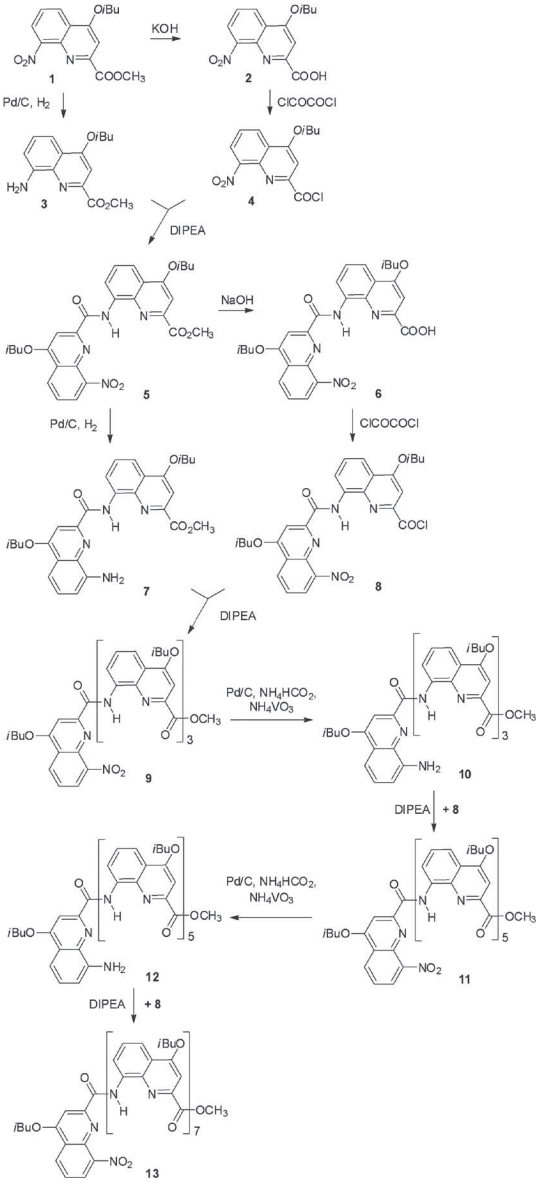

Figure 1 | Synthesis of oligoamides of 6-amino-4-isobutoxy-2-quinolinecarboxylic acid from nitro ester **1**.

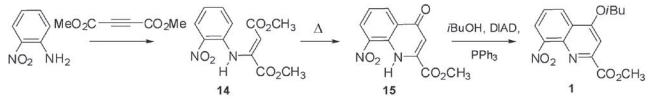

Figure 2 | Preparation of methyl-4-isobutoxy-8-nitroquinoline-2-carboxylate **1** via dimethyl 2-(2-nitrophenylamino)-fumarate **14**.

into tertiary amides[36]. Folding has also been found to promote some (undesired) reactions that are specific to aromatic backbones[37], resulting in further potential hurdles. At the time we first described the synthesis of oligoamides of 6-amino-4-isobutoxy-2-quinolinecarboxylic acid[38], a number of issues and possible pitfalls had been overlooked, including possible chlorination and hydroxylation of the backbones, as well as anhydride formation. After multiple years of incremental improvements, we are now in a position to report an optimized and reliable protocol, which stands, to our knowledge, as the first large-scale and chromatography-free preparation of a foldamer.

Experimental design

The procedures described below have been designed to solve a number of issues pertaining to the formation of by-products and to facilitate purification procedures in order to avoid chromatographic separation of the products if possible and to allow for scaling up of the reactions. For example, it was found that chlorination of aromatic backbones occurs when long oligomers are refluxed in thionyl chloride ($SOCl_2$), even when care is taken to distill it before reaction[37]. The use of this reagent should therefore be avoided, and it has successfully been replaced by oxalyl chloride. It was also found that when nitro groups are not fully hydrogenated some remaining hydroxylamine[39] intermediate contaminates the following coupling step, giving rise to a rearranged hydroxy-aromatic product, which is difficult to remove. Careful monitoring and, where needed, the use of ammonium formate as a source of hydrogen and ammonium metavanadate[39] as a cocatalyst alleviate these difficulties. Finally, an iterative synthetic strategy is proposed here in order to replace the more convergent scheme initially described[38]. In the convergent scheme, two dimers were coupled to produce a tetramer and two tetramers were coupled to produce an octamer. In the iterative strategy described here, dimers are successively added to the tetramer to produce a hexamer and then an octamer. This approach avoids the difficult saponification of the tetramer ester and the potential formation of an unwanted anhydride by-product of the tetramer acid during its activation (which complicates purification by making chromatography necessary and limits the scale up of these reactions). In contrast, when short segments such as a dimer are added to the N terminus of a tetramer or a hexamer, high coupling yields are achieved and excess reagent is easily separated from the product by simple recrystallization. Because chromatography is avoided, this approach can be scaled up to multiple grams.

These optimized procedures and the troubleshooting table are directly usable or easily adaptable to monomers and oligomers bearing other side chains than isobutoxy groups, which considerably broadens the scope of this protocol. The Mitsunobu conditions used to introduce the side chain (synthesis of **1**, Steps 1–24 of the PROCEDURE (**Fig. 2**); ^{1}H NMR spectra for compounds **1–15** can be found in **Supplementary Figs. 1–15**) are high yielding and of general use with diverse primary aliphatic alcohols[27,32]. Mitsunobu reactions generate by-products, notably triphenylphosphine oxide, that are difficult to remove by chromatography. For large-scale syntheses, it is crucial that efficient recrystallization conditions be found to purify the product. Methanol was an effective crystallization solvent in all cases encountered so far[27,32]. Alternatively, side chains could be introduced using a Williamson alkylation with strong electrophiles such as benzyl bromide or bromo-acetate derivatives[33].

When the side chains contain acid-labile functions, such as *tert*-butyl ester[30,33] or *tert*-butyl carbamate[27,30], activation of the C-terminal acid function as an acid chloride should be carried out under neutral conditions. For this purpose, oxalyl chloride is not appropriate, as its reaction generates hydrochloric acid[34]. Oxalyl chloride can be effectively replaced by 1-chloro-*N*,*N*-2-trimethyl-1-propenylamine (Ghosez's reagent)[40] in each acid-activation step[27,32,33]. 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent) has properties similar to those of Ghosez's reagent and has been shown to be a valid activating agent in other series of aromatic

amide foldamers bearing acid-labile residues[34], but it has not been tested to prepare oligomers of 8-amino-2-quinolinecarboxylic acid. All side chain–protecting groups can be removed at once in a final acid treatment with trifluoroacetic acid[27,33].

When the side chains contain base-labile functions, the C-terminal methyl ester should be replaced by a 2-trimethylsilyl-ethyl ester, which can then be conveniently and quantitatively removed using orthogonal (nonbasic) deprotection conditions in the presence of tetrabutyl ammonium fluoride[30]. The need for this change of main-chain ester arose with some *tert*-butyl ester functions in the side chains, which were unexpectedly found to be as base labile as the methyl ester of the main chain[33].

MATERIALS

REAGENTS

! CAUTION It should be noted that dimethyl acetylene dicarboxylate, diphenyl ether, hydrochloric acid and oxalyl chloride are toxic and harmful on inhalation, ingestion or on skin contact. Dimethyl acetylene dicarboxylate is an irritant and very hazardous in case of skin contact, eye contact or ingestion. It is light sensitive and should be stored in a dark place. Diphenyl ether is an irritant and has an unpleasant smell. Concentrated hydrochloric acid forms acidic mists. Both the mist and the solution have a corrosive effect on human tissue, with the potential to damage respiratory organs, eyes, skin and intestines. Oxalyl chloride reacts with water to produce HCl gas, which creates foaming and a possible rise of internal pressure. The addition of oxalyl chloride must be slow and carefully controlled. HCl gas should be allowed out of the flask through an open vacuum adapter. Palladium on carbon may ignite on exposure to air, particularly when it contains adsorbed hydrogen; it readily causes ignition of flammable solvents in the presence of air. Palladium on carbon should always be handled under an inert atmosphere and reaction vessels should be flushed with inert gas (preferably argon) before the catalyst is added. Dry catalyst should never be added to an organic solvent in the presence of air. Palladium on carbon recovered from reactions by filtration requires careful handling because it is usually saturated with hydrogen and will ignite spontaneously on exposure to air. The filter cake should never be allowed to dry and the moist material should be added to a large quantity of water and disposed of properly. These compounds should be handled in a well-ventilated fume hood by wearing personal protective equipment such as rubber or PVC gloves, protective eye goggles, chemical-resistant clothing and shoes to minimize risks.

▲ CRITICAL All procedures should be performed in a fume hood. The solid and liquid waste products generated should be disposed of appropriately, as defined locally. The solvents used in the reactions should be of the highest quality available. When an anhydrous solvent is to be used, it is indicated clearly in the PROCEDURE. Anhydrous solvents are dried according to the methods indicated, or they are purchased in anhydrous form as indicated. The solvents used in the extraction and workup phases should be of reagent quality or better.

- 2-Methyl propanol (Alfa Aesar, cat. no. 036643)
- 2-Nitroaniline (Sigma-Aldrich, cat. no. N9780)
- Anhydrous $MgSO_4$
- Argon
- CaH_2
- Celite (VWR international, cat. no. 22552-90)
- Chloroform (Sigma-Aldrich, cat. no. 25693)
- Chloroform-*d* (Euriso-top, cat. no. D006H)
- Cyclohexane
- Dichloromethane (Sigma-Aldrich, cat. no. 24233)
- Diisopropyl azodicarboxylate (Sigma-Aldrich, cat. no. 225541)
- *N,N*-Diisopropylethylamine (DIPEA) (Sigma-Aldrich, cat. no. D12,580-6)
- Dimethyl acetylene dicarboxylate (Sigma-Aldrich, cat. no. D138401)
- Diphenyl ether (Sigma-Aldrich, cat. no. P24101)
- Ethylacetate
- Hydrochloric acid (aqueous solution, minimum 37% (wt/wt))
- Hydrogen
- Isobutyl alcohol
- Methanol
- $NaHCO_3$
- Nitrogen
- Oxalyl chloride (Sigma-Aldrich, cat. no. O8801)
- Pd/C 10% (Alfa Aesar, cat. no. A12012)
- Silica gel
- Sodium chloride
- Tetrahydrofuran (THF) (Sigma-Aldrich, cat. no. 178810)
- Toluene (Sigma-Aldrich, cat. no. 244511)
- Triphenylphosphine (Alfa Aesar, cat. no. A14089)

EQUIPMENT

- Adapter
- Balloons
- Beaker (50 ml)
- Cannula
- Erlenmeyer flasks (200–3,000 ml)
- Sintered glass filter funnels (porosity grades 1 and 3)
- Freezer (−18 °C)
- Heating mantle for 1,000-ml round-bottomed flask
- Ice
- Mechanical stirrer assembly
- Oil bath
- Plastic syringes (5, 10 and 20 ml)
- Needles
- Round-bottomed flasks and two-necked round-bottom flasks (250–1,000 ml)
- Rotary evaporator (Büchi, model R-3000); vacuum pump (Büchi, model V-500); vacuum controller (Büchi, model V-800)
- Reflux condenser
- Stoppered vacuum adapters
- Teflon-coated magnetic stir bars
- Thermometers covering the range of −18 to 260 °C
- UV lamp
- Vacuum line with a dual manifold for both vacuum and nitrogen

REAGENT SETUP

Dichloromethane Dry dichloromethane is obtained by filtration through activated alumina using a dedicated purification system[41] and should be used immediately.

Chloroform-*d* Chloroform should be passed over a short pad of alumina to remove traces of HCl. Traces of acid give unrepresentative NMR spectra, especially in the case of the amine oligomers. It can be stored for 1 month in the dark at 7 °C.

THF Dry THF is obtained by filtration through activated alumina using a dedicated purification system[41] and should be used immediately.

Toluene Dry toluene is obtained by filtration through activated alumina using a dedicated purification system[41] and should be used immediately.

***N,N*-diisopropylethylamine** It should be freshly distilled over CaH_2 to remove traces of water and used immediately.

EQUIPMENT SETUP

Thin-layer chromatography (TLC) Prepare two TLC tanks: one with 100% CH_2Cl_2 and the other with CH_2Cl_2/methanol (95:5, vol/vol).

Dry syringes and needles Plastic syringes and needles directly used from the packaging are considered dry. When they are reused, they should be cleaned with acetone and dried under vacuum (at least for 1 h).

PROCEDURE

▲ CRITICAL The first 24 steps describe the synthesis of the monomer (**1**) as shown in **Figure 2**.

Preparation of dimethyl 2-(2-nitrophenylamino)-fumarate (14) ● TIMING 42 h

1| Place a 1-liter round-bottomed flask equipped with a Teflon-coated magnetic stir bar in an oil bath.

2| Dissolve 2-nitroaniline (40.0 g, 290 mmol) in 400 ml of methanol in the reaction flask.

3| By using a syringe, add dimethyl acetylene dicarboxylate (35.6 ml, 290 mmol) portionwise to the solution while stirring.

4| Equip the reaction flask with a reflux condenser and increase the temperature of the oil bath to 65 °C. Stir the reaction mixture at 65 °C for 24 h.

5| Remove the oil bath and leave the reaction mixture to cool down to room temperature (20–25 °C). Reduce the solvent volume to 70 ml using a rotary evaporator.

6| Store the flask at −18 °C for 12 h, during which time yellow prisms are formed.

7| Collect the yellow crystals by filtration and wash them with cold methanol (300 ml at −18 °C). Dry the crystals under vacuum for 5 h. A typical yield is 75% (i.e., 60.0 g), and this reaction procedure can be easily scaled up to 100 g. For analytical data, see **Supplementary Figure 14** and ref. 42.

Preparation of methyl-8-nitro-(1H)-4-quinolinone-2-carboxylate (15) ● TIMING 8 h 45 min

8| Place a 1-liter round-bottomed flask in a heating mantle (no stirrer is needed).

9| If needed, warm a bottle of diphenyl ether (melting point 25–26 °C) in a water bath to ensure that it is in a liquid state. Measure and add 470 ml of diphenyl ether into the reaction flask, and then heat it to its boiling point at 260 °C without any reflux condenser or stir bar. Monitor the temperature using an appropriate thermometer.

10| Add dimethyl 2-(2-nitrophenylamino)-fumarate **14** (25.0 g, 89.3 mmol) at once to the boiling mixture by means of a glass funnel.

11| Heat the reaction mixture further at 260 °C, leaving the reaction flask open without a reflux condenser to allow methanol vapors out of the reaction mixture. Monitor the reaction by TLC (silica gel, 100% CH_2Cl_2). Visualize the developed TLC with a UV lamp at λ = 254 nm. Fumarate **14** and quinolinone **15** have retardation factors of 0.5 and 0.1, respectively. Continue the reaction until the spot of the starting material has almost disappeared. This takes between 12 and 15 min.
▲ CRITICAL STEP Longer heating time decreases the product purity and the yield of this reaction.

12| Remove the heating mantle and leave the flask to cool down in air to ~50 °C.
! CAUTION Use appropriate equipment and protection for the high-temperature reaction. Do not use a cold bath, as the temperature shock may break the flask.

13| Add cyclohexane or petroleum ether (500 ml) to the cooled reaction mixture and transfer the resulting suspension to a 2-liter Erlenmeyer flask containing 1 liter of cyclohexane or petroleum ether.

14| Isolate the resulting precipitate by filtration through a 500-ml sintered glass filter funnel (porosity grade 1) and wash it thoroughly with 1.5 liters of cyclohexane or petroleum ether to remove the diphenyl ether.
! CAUTION After workup and glassware cleaning, diphenyl ether–containing wastes should be stored in appropriate closed containers in well-ventilated fume hoods.

15| Dry the brownish solid under vacuum for 5 h (typical yield of 75%, i.e., 16.6 g). For analytical data, see **Supplementary Figure 15** and ref. 42.

Synthesis of methyl-4-isobutoxy-8-nitroquinoline-2-carboxylate (1) ● TIMING 32.5 h

16| Dry a 250-ml two-necked round-bottomed flask containing a Teflon-coated magnetic stir bar, a stoppered vacuum adapter and a cannula at 120 °C in an oven (at least for 1 h).

17| Add methyl-8-nitro-(1H)-4-quinolinone-2-carboxylate **15** (16.0 g, 64.5 mmol) and triphenylphosphine (18.6 g, 71.0 mmol) into the reaction flask. Close the flask hermetically with a rubber septum on one neck and the stoppered vacuum adapter on the other neck. Remove air by briefly placing the flask under vacuum through the adapter, close the vacuum adapter and fill the flask back with nitrogen using the vacuum line with a dual manifold or with a nitrogen-filled balloon. Repeat this operation twice and leave the flask under positive nitrogen pressure. Add isobutyl alcohol (6.5 ml, 71.0 mmol) into the flask using a syringe through the septum and cool it down to 0 °C by placing it in an ice bath.
! CAUTION Do not leave the reaction flask under vacuum for long, as triphenylphosphine slowly sublimates.
! CAUTION Alternating vacuum and nitrogen back-filling should be carried out carefully to avoid blowing away powdery solids.
▲ CRITICAL STEP Moisture should be prevented from entering the reaction flask. Therefore, the reaction flask should always be under positive nitrogen pressure.

18| Suspend the reagents in 120 ml of dry THF, which is added through a dry cannula into the reaction flask.

19| By using a dry syringe, deliver diisopropyl azodicarboxylate (DIAD) (15.9 ml, 71.0 mmol) to the solution and stir the reaction mixture further at 0 °C for 30 min. The suspension will become a clear solution upon the addition of DIAD.

20| Remove the ice bath. Let the reaction mixture warm up to room temperature and stir it for 12 h.

21| Remove the solvents using a rotary evaporator.

22| Dissolve the crude mixture in 80 ml of cold methanol (−18 °C) and place the solution at −18 °C for 12 h. The product will crystallize upon addition of cold methanol.

23| Collect the yellow crystals by filtration through a 125-ml sintered glass filter funnel (porosity grade 3) and wash them three times, each with 10 ml of cold methanol (−18 °C).

24| Dry compound **1** under vacuum for 5 h (typical yield 71%, i.e., 14.0 g). For analytical data of monomer **1**, see **Supplementary Figure 1** and ref. 38.

Preparation of the dimer, tetramer, hexamer and octamer

25| For the coupling reaction that results in the formation of the dimer, tetramer, hexamer or octamer, you will need two main starting materials: an amine prepared by reducing the N-terminal nitro group and an acyl chloride resulting from the saponification of a C-terminal methyl ester, followed by reaction with oxalyl chloride (**Fig. 1** and options A–D below). The amine and the acyl chloride are coupled using DIPEA by following Steps 26–37.

(A) Preparation of the dimer

(i) Prepare monomer amine **3** as described in **Box 1** and check its purity (**Fig. 3**).
(ii) Prepare monomer acid chloride **4** (23.4 mmol, 1.05 equiv.) as described in **Box 2** and check its purity (**Fig. 4**).
▲ CRITICAL STEP Monomer acid chloride **4** cannot be stored and should be prepared and used immediately for coupling.
▲ CRITICAL STEP Moisture should be prevented from entering the reaction flask. Therefore, the flask should always be under positive nitrogen pressure.
(iii) Follow Steps 26–37.

(B) Preparation of the tetramer

(i) Prepare dimer amine **7** as described in **Box 1** and check its purity (**Fig. 5**).
(ii) Prepare dimer acid chloride **8** (5.64 mmol, 1.08 equiv.) as described in **Box 3** and check its purity (**Fig. 6**).
▲ CRITICAL STEP Dimer acid chloride **8** cannot be stored and should be prepared and used immediately for coupling.
▲ CRITICAL STEP Moisture should be prevented from entering the reaction flask. Therefore, the flask should always be under positive nitrogen pressure.
(iii) Follow Steps 26–37.

(C) Preparation of the hexamer

(i) Prepare tetramer amine **10** as described in **Box 4** and check its purity (**Fig. 7**).
(ii) Starting from 2.80 g of dimer acid **6** (5.26 mmol, 1.07 equiv.), prepare dimer acid chloride **8**, as described in **Box 2**, and check its purity (**Fig. 6**).
▲ CRITICAL STEP Moisture should be prevented from entering the reaction flask. Therefore, the flask should always be under positive nitrogen pressure.
(iii) Follow Steps 26–37.

Box 1 | Preparation of monomer amine 3 and dimer amine 7

The preparation of monomer amine **3** and dimer amine **7** is carried out using Pd/C-catalyzed hydrogenation, and it differs from the preparation of tetramer amine **10** and hexamer amine **12** shown in **Box 4**.

PROCEDURE

1. Equip a 500-ml two-necked round-bottomed flask with a Teflon-coated magnetic stir bar. Close one neck with a septum.
2. Place monomer **1** (6.80 g, 22.4 mmol) into the reaction flask and add 150 ml of ethyl acetate. Flush with argon and then add Pd/C (10%) catalyst (680 mg) to the solution. Rinse the wall of the flask with 10 ml of ethyl acetate. Place dimer **5** (2.90 g, 5.31 mmol) into the reaction flask and add 340 ml of ethyl acetate. Flush with argon and then add Pd/C (10%) catalyst (290 mg) to the solution. Rinse the wall of the flask with 10 ml of ethyl acetate.
3. Fill a balloon with hydrogen and place the reaction mixture under a hydrogen atmosphere by alternatively purging the flask under vacuum, shaking it and filling the flask with hydrogen (three times). Continue stirring vigorously under hydrogen atmosphere for 12 h at room temperature for the reduction of monomer **1** and at 50 °C for the reduction of dimer **5**.

▲ **CRITICAL STEP** Ensure that a balloon pressure of hydrogen is maintained during the entire reaction time. If the hydrogen balloon shrinks, replace it by a new, freshly filled balloon. Vigorous stirring is required.

4. Check the reaction progress by ^{1}H NMR spectroscopy after 12 h before proceeding. By using a syringe through the septum, take out a 0.05-ml aliquot of the reaction solution and place it into an NMR tube. Remove the solvent using a high-vacuum line. Dissolve the residue in 0.5 ml of $CDCl_3$ and record a ^{1}H NMR spectrum. Confirm the quantitative amine formation by the singlet at 5.11 p.p.m. for monomer amine **3** (**Fig. 3**) and at 5.55 p.p.m. for dimer amine **7** (**Fig. 5**).

▲ **CRITICAL STEP** If the reduction is incomplete, intermediates of the reduction process are observed in the ^{1}H NMR spectrum of monomer amine **3** and dimer amine **7**. The reduction should be continued for another 24 h by applying a freshly filled hydrogen balloon.

? TROUBLESHOOTING

5. Dilute the reaction mixture with 50 ml of CH_2Cl_2. Filter the reaction mixture through a 125-ml sintered glass filter funnel (porosity grade 3) filled with a pad of packed Celite to remove the Pd/C. Rinse the Celite with 50 ml of CH_2Cl_2 to collect all yellow products.
6. Evaporate the filtrate using a rotary evaporator.
7. Dry the residue under vacuum for 5 h. The bright yellow monomer amine **3** and dimer amine **7** (typical yield 99 %, i.e., 6.10 g of monomer amine **3**, i.e., 2.70 g dimer amine **7**) are used without further purification in the coupling reactions (Steps 25–37 in the PROCEDURE). For analytical data, see the **Supplementary Figure 3** (monomer amine **3**), **Supplementary Figure 7** (dimer amine **7**) and ref. 38. Compounds **3** and **7** can be stored at room temperature and in the dark for at least 3 months.

▲ **CRITICAL STEP** The amine should be thoroughly dried, as the coupling reaction is moisture sensitive.

● **TIMING**

Preparation of monomer amine 3 and dimer amine 7: 19 h

Steps 1–3: 12 h

Steps 4–7: 7 h

(D) Preparation of the octamer

(i) Prepare hexamer amine **12** as described in **Box 4** and check its purity (**Fig. 8**).

(ii) Starting from 2.50 g of dimer acid **6** (4.70 mmol, 1.12 equiv.), prepare dimer acid chloride **8** as described in **Box 2** and check its purity (**Fig. 6**).

▲ **CRITICAL STEP** Moisture should be prevented from entering the reaction flask. Therefore, the flask should always be under positive nitrogen pressure.

(iii) Follow Steps 26–37.

Coupling using DIPEA

26| Dry a two-necked round-bottomed flask containing a Teflon-coated magnetic stir bar, a stoppered vacuum adapter and a cannula at 120 °C in an oven (at least for 1 h).

Synthesis of:	Size of round-bottomed flask (ml)
Dimer **5**	500
Tetramer **9**	250
Hexamer **11**	250
Octamer **13**	500

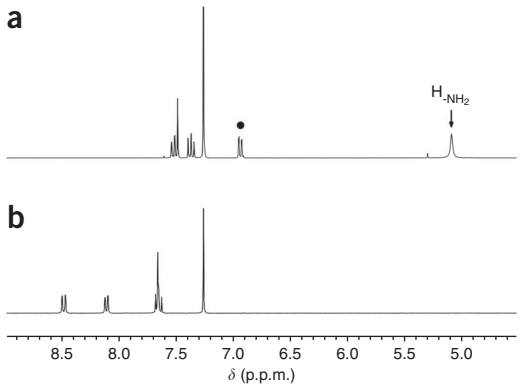

Figure 3 | Preparation of the monomer. (**a**,**b**) Part of the ^{1}H-NMR spectra (25 °C, $CDCl_3$, 300 MHz) of monomer amine **3** (**a**) and monomer **1** (**b**). Characteristic peaks are marked with a dot and an arrow.

Box 2 | Preparation of monomer acid chloride 4

The preparation of monomer acid chloride **4** is easily carried out in two steps from monomer **1** (**Fig. 1**). It differs slightly from the preparation of dimer acid chloride **8** described in **Box 2**.

Additional materials
Potassium hydroxide

PROCEDURE
1. Equip a 1-liter two-necked round-bottomed flask with a mechanical stirrer.
2. Place monomer **1** (7.20 g, 23.7 mmol) into the reaction flask and add 290 ml of 1,4-dioxane and 30 ml of distilled water.
3. Crush KOH pellets in a mortar and add KOH powder (3.30 g, 58.9 mmol) to the solution and stir the reaction mixture at room temperature for about 24 h (see next step).
4. Monitor the reaction by TLC (silica gel, CH_2Cl_2/methanol 95:5, vol/vol). Visualize the developed TLC with a UV lamp at λ = 254 nm. Monomer ester **1** and monomer acid **2** have retardation factors of 0.7 and 0.1, respectively. Continue stirring until the spot of the starting material disappears on a TLC plate.
5. When the reaction is complete, add 100 ml of aqueous citric acid (5%, wt/wt) to the reaction mixture. A slightly acidic solution should be obtained, which can be checked with a pH paper (pH ~5).
6. Remove the 1,4-dioxane using a rotary evaporator.
7. Add 250 ml of CH_2Cl_2 into the flask. Transfer the solution into a 1-liter separation funnel and separate the layers. Wash the organic layer with 200 ml of distilled water and 200 ml of saturated aqueous NaCl. Combine the aqueous layers and extract them twice with 100 ml of CH_2Cl_2. Combine the organic layers and dry them over $MgSO_4$ for 1 h. Filter the mixture through a 125-ml sintered glass filter funnel (porosity grade 3). Rinse the solid residue three times, each with 5 ml of CH_2Cl_2.
8. Remove the solvent using a rotary evaporator.
9. Dry the residue under vacuum for 5 h. The light yellow product (typical yield 99%, i.e., 6.80 g) is used without further purification in the next step. For analytical data of monomer acid **2**, see **Supplementary Figure 2** and ref. 38.
■ **PAUSE POINT** Monomer acid **2** can be stored at room temperature for at least 1 year.
10. Dry a 250-ml two-necked round-bottomed flask containing a Teflon-coated magnetic stir bar, a stoppered vacuum adapter and a cannula at 120 °C in an oven (at least for 1 h).
11. Place monomer acid **2** (6.80 g, 23.4 mmol) into the dry reaction flask and close the flask hermetically with a rubber septum on one neck and the vacuum adapter on the other neck. Dry the starting material via the vacuum adapter using a high-vacuum line for 1 h.
▲ **CRITICAL STEP** The starting material should be thoroughly dried, as the reaction is moisture sensitive.
12. Turn off the vacuum adapter and detach it from the vacuum line. Fill the flask back with nitrogen using a needle through the septum. Open the vacuum adapter and allow a slow flow of nitrogen out of the flask.
▲ **CRITICAL STEP** Moisture should be prevented from entering the reaction flask. Therefore, the reaction flask should always be under positive nitrogen pressure.
13. By using a dry syringe through the septum, deliver 45 ml of anhydrous CH_2Cl_2 into the reaction flask and cool it to 0 °C by placing the flask in an ice bath.
14. By using a dry syringe through the septum, slowly deliver oxalyl chloride (10.0 ml, 117 mmol) into the reaction flask. Remove the ice bath and stir the reaction mixture at room temperature for 2 h.
15. Check the reaction progress by ^{1}H NMR spectroscopy before proceeding. By using a dry syringe, take out a 0.05-ml aliquot of the reaction solution and place it into an NMR tube. Remove the solvent using a high-vacuum line. Dissolve the residue in 0.5 ml of $CDCl_3$ and record a ^{1}H NMR spectrum. Confirm the quantitative acid chloride formation by monitoring a sharp singlet at 7.55 p.p.m. (**Fig. 4**).
16. Remove the solvent and residual oxalyl chloride without heating using a vacuum line equipped with a large liquid-nitrogen trap. Allow the residual solid to dry under high vacuum for 5 h. If high vacuum is unavailable, add some anhydrous toluene and evaporate all volatiles again without heating, using a vacuum line equipped with a large liquid-nitrogen trap. Repeat this procedure twice.
17. Turn off the vacuum and fill the flask back with nitrogen using the dual manifold of the vacuum line or with a nitrogen-filled balloon. For a ^{1}H NMR spectrum of monomer acid chloride **4**, see **Supplementary Figure 4**.
▲ **CRITICAL STEP** Oxalyl chloride should be thoroughly removed as mentioned in step 16, as residues will react with amines during the subsequent coupling steps, generating impurities that can be difficult to separate.
▲ **CRITICAL STEP** The freshly obtained acid chloride **4** cannot be stored and is used at once without further purification for the preparation of dimer **9** (Steps 25–37 in the PROCEDURE).
▲ **CRITICAL STEP** Moisture should be prevented from entering the reaction flask, as the acid chloride is moisture sensitive. Therefore, the reaction flask should always be either under vacuum or under positive nitrogen pressure.

● **TIMING**
Preparation of monomer acid chloride 4: 44 h
Steps 1–4: 24 h
Steps 5–9: 10 h
Steps 10 and 11: 2 h
Steps 12–15: 3 h
Step 16: 5 h

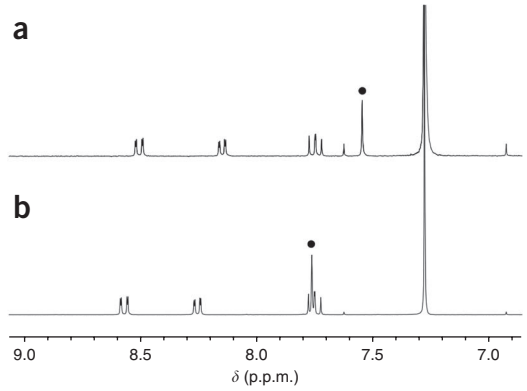

Figure 4 | Preparation of the monomer acid chloride. (**a**,**b**) Part of the ^{1}H NMR spectra (25 °C, $CDCl_3$, 300 MHz) of monomer acid chloride **4** (**a**) and monomer acid **2** (**b**). Characteristic peaks are marked by dots.

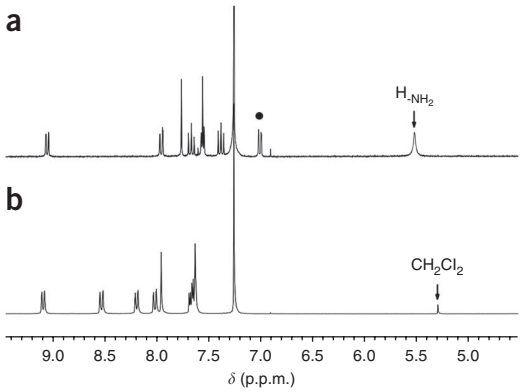

Figure 5 | Preparation of the dimer amine. (**a**,**b**) Part of the ^{1}H-NMR spectra (25 °C, $CDCl_3$, 300 MHz) of dimer amine **7** (**a**) and dimer **5** (**b**). Characteristic peaks are marked with a dot and an arrow.

27| Add dry amine into the dry reaction flask, and close it hermetically with a rubber septum on one neck and the vacuum adapter on the other neck. Place the flask under vacuum via the vacuum adapter.

Amine starting material	Mass to be added (g)	Amount (mmol)
Monomer amine **3**	6.10	22.3
Dimer amine **7**	2.70	5.23
Tetramer amine **10**	4.90	4.83
Hexamer amine **12**	6.60	4.44

Box 3 | Preparation of dimer acid chloride 8

The preparation of dimer acid chloride **8** from dimer methyl ester **5** is easily carried out in two steps (**Fig. 1**). It differs slightly from the preparation of monomer acid chloride **4** described in **Box 2**.

Additional materials
Sodium hydroxide

PROCEDURE
1. Equip a 1-liter round-bottomed flask with a large Teflon-coated magnetic stir bar.
2. Add dimer **5** (8.90 g, 16.3 mmol) into the reaction flask and add 270 ml of THF and 90 of ml methanol.
3. Crush NaOH pellets in a mortar and add NaOH powder (2.00 g, 50.0 mmol) into the reaction flask and stir the reaction mixture at room temperature for 8 h.
4. Monitor the reaction by TLC (silica gel, CH_2Cl_2/methanol 95:5, vol/vol). Visualize the developed TLC with a UV lamp at λ = 254 nm. Dimer ester **5** and dimer acid **6** have retardation factors of 0.9 and 0.6, respectively. Continue stirring until the spot of the starting material disappears on a TLC plate.
5. When the reaction is complete, add 80 ml of an aqueous citric acid solution (5%, wt/wt) to the reaction mixture. A slightly acidic solution should be obtained, which can be checked with a pH paper (pH ~5).
6. Remove the THF and methanol using a rotary evaporator.
7. Add 300 ml of CH_2Cl_2 into the flask. Transfer the solution into a 1-liter separation funnel and separate the layers. Wash the organic layer with 200 ml of distilled water and 200 ml of saturated aqueous NaCl. Combine the aqueous layers and extract them twice with 100 ml of CH_2Cl_2. Combine the organic layers and dry them over $MgSO_4$ for 1 h. Filter the mixture through a 125-ml sintered glass filter funnel (porosity grade 3). Rinse the residue three times, each with 5 ml of CH_2Cl_2.
8. Remove the solvent using a rotary evaporator.
9. Dry the residue under vacuum for 5 h. The light yellow product (typical yield 99% i.e., 8.60 g) is used without further purification in the next step. For analytical data of dimer acid **6**, see **Supplementary Figure 6** and ref. 38.

■ **PAUSE POINT** Dimer acid **6** can be stored at room temperature for at least 1 year.

(continued)

Box 3 | (continued)

10. Dry a 250-ml two-necked round-bottomed flask containing a Teflon-coated magnetic stir bar, a stoppered vacuum adapter and a cannula at 120 °C in an oven (at least for 1 h).
11. Place dimer acid **6** (3.00 g, 5.64 mmol) into the dry reaction flask and close the flask hermetically with a rubber septum on one neck and the vacuum adapter on the other neck. Dry the starting material via the vacuum adapter using a high-vacuum line for 1 h.
▲ **CRITICAL STEP** The exact amount of dimer acid **6** should be adjusted as described in the PROCEDURE depending on whether it is used to prepare the tetramer, the hexamer or the octamer in order to amount to 1.07–1.12 equivalent with respect to the amine to which it is coupled.
▲ **CRITICAL STEP** The starting material should be thoroughly dried, as the reaction is moisture sensitive.
12. Turn off the vacuum adapter and detach it from the vacuum line. Fill the flask back with nitrogen using a needle through the septum. Open the vacuum adapter and allow a slow flow of nitrogen out of the flask.
▲ **CRITICAL STEP** Moisture should be prevented from entering the reaction flask. Therefore, the reaction flask should always be under positive nitrogen pressure.
13. By using a dry syringe through the septum, deliver 45 ml of anhydrous CH_2Cl_2 into the reaction flask and cool the solution down to 0 °C by placing the flask in an ice bath.
14. By using a dry syringe through the septum, deliver oxalyl chloride (2.4 ml, 28.0 mmol) slowly into the reaction flask. Remove the ice bath and stir the reaction mixture at room temperature for 2 h.
15. Check the reaction progress by ^{1}H NMR spectroscopy before proceeding. By using a dry syringe, take out a 0.05-ml aliquot of the reaction solution and place it into an NMR tube. Remove the solvent using a high-vacuum line. Dissolve the residue in 0.5 ml of $CDCl_3$ and record an ^{1}H NMR spectrum. Confirm the quantitative acid chloride formation by the sharp singlet at 7.54 p.p.m. (**Fig. 6**).
16. Remove the solvent and residual oxalyl chloride without heating under a vacuum line equipped with a large liquid-nitrogen trap. Allow the residual solid to dry under high vacuum for 5 h. If high vacuum is unavailable, add some anhydrous toluene and evaporate all volatiles without heating using a vacuum line equipped with a large liquid-nitrogen trap. Repeat this procedure twice.
17. Turn off the vacuum and fill the flask back with nitrogen using the dual manifold of the vacuum line or with a nitrogen-filled balloon. For a ^{1}H NMR spectrum of dimer acid chloride **8** see **Supplementary Figure 8**.
▲ **CRITICAL STEP** The freshly obtained acid chloride **8** cannot be stored and is used at once without further purification in the coupling reactions (Steps 25–37 in the PROCEDURE).
▲ **CRITICAL STEP** Moisture should be prevented from entering the reaction flask, as the acid chloride is moisture sensitive. Therefore, the reaction flask should always be either under vacuum or under positive nitrogen pressure.
▲ **CRITICAL STEP** Oxalyl chloride should be thoroughly removed, as residues will react with amines during the subsequent coupling steps, generating impurities that can be difficult to separate.

● **TIMING**
Preparation of dimer acid chloride 8: 28 h
Steps 1–4: 8 h
Steps 5–9: 10 h
Steps 10 and 11: 2 h
Steps 12–15: 3 h
Step 16: 5 h

28| Turn off the vacuum and fill the flask back with nitrogen using the dual manifold of the vacuum line or with a nitrogen-filled balloon.
▲ **CRITICAL STEP** Moisture should be prevented from entering the reaction flask. Therefore, the reaction flask should always be under positive nitrogen pressure.

29| By using a dry syringe through the septum, deliver dry CH_2Cl_2 to dissolve the amine.

Amine starting material	Volume of dry CH_2Cl_2 (ml)
Monomer amine **3**	55
Dimer amine **7**	10
Tetramer amine **10**	20
Hexamer amine **12**	20

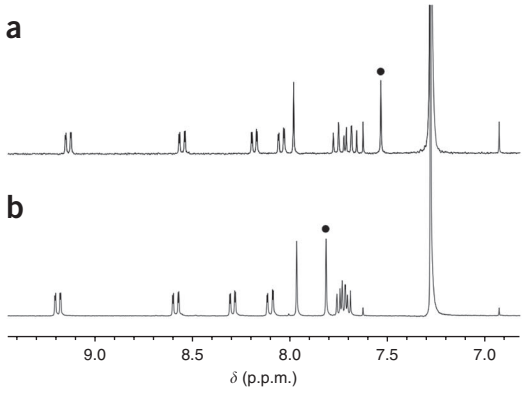

Figure 6 | Preparation of the dimer acid chloride. (**a**,**b**) Part of the ^{1}H-NMR spectra (25 °C, $CDCl_3$, 300 MHz) of dimer acid chloride **8** (**a**) and dimer acid **6** (**b**). Characteristic peaks are marked by dots.

Box 4 | Preparation of tetramer amine 10 and hexamer amine 12

The preparation of tetramer amine **10** and hexamer amine **12** is carried out using Pd/C-catalyzed hydrogenation with ammonium formate as a hydrogen source, and differs from the preparation of monomer amine **3** and dimer amine **7** shown in **Box 1**.

Additional materials
Ammonium formate (Alfa Aesar, cat. no. A10699)
Ammonium metavanadate[39] (Sigma-Aldrich, cat. no. 398128)

PROCEDURE
1. Place a 500-ml two-necked round-bottomed flask equipped with a Teflon-coated magnetic stir bar in an oil bath. Close one neck with a septum.
2. Place tetramer **9** (5.20 g, 5.05 mmol) into the reaction flask and add 240 ml of ethyl acetate and 60 ml of ethanol. Flush with argon and then add the Pd/C (10%) catalyst (525 mg) and ammonium metavanadate (262 mg) into the reaction flask.
Place hexamer **11** (7.00 g, 4.62 mmol) into the reaction flask and add 220 ml of ethyl acetate and 55 ml of ethanol. Flush with argon and then add the Pd/C (10%) catalyst (700 mg) and ammonium metavanadate (350 mg) into the reaction flask.
3. Equip the reaction flask with a reflux condenser capped with a balloon filled with argon, and heat the reaction solution to 95 °C.
4. Prepare an aqueous solution of ammonium formate in a 50-ml beaker. Add 15.7 g of NH_4HCO_2 (250 mmol) in 12 ml of water for the tetramer **9** reduction; add 14.3 g of NH_4HCO_2 (230 mmol) in 11 ml of water for the hexamer **11** reduction.
5. By using a syringe through the septum, slowly add a fifth of the ammonium formate solution to the hot reaction mixture. Repeat this operation four times every 10 min.
6. Continue stirring at 95 °C for 12 h under an inert atmosphere.
! CAUTION During refluxing, ammonium formate can solidify at the bottom of the condenser and clog it. Pressure may then build inside the reaction flask, making it dangerous when opening the system. Make sure to use a large neck and the appropriate condenser size.
7. Monitoring the reaction by ^{1}H NMR spectroscopy may allow to observe reaction completion significantly earlier than prescribed. By using a syringe through the septum, take out a 0.05-ml aliquot from the reaction solution and place it into an NMR tube. Remove the solvent using a high-vacuum line. Dissolve the residue in 0.5 ml of $CDCl_3$ and record a ^{1}H NMR spectrum. Confirm the quantitative amine formation by monitoring the doublet of doublets at 6.00 p.p.m. for tetramer amine **10** (**Fig. 7**) and at 5.85 p.p.m. for hexamer amine **12** (**Fig. 8**).
! CAUTION Be careful with the high reaction temperature when taking out the aliquot for the control ^{1}H NMR.
▲ CRITICAL STEP If the reduction is still incomplete after 12 h, intermediates are observed by ^{1}H NMR (**Fig. 11a**). To eliminate such species and facilitate further synthesis, the reduction should be continued by adding more of the ammonium formate solution (5.00 g of NH_4HCO_2 in 5 ml of H_2O) and by further stirring at 95 °C. The reduction will continue and yield quantitatively the amine as indicated by ^{1}H NMR spectroscopy.
? TROUBLESHOOTING
8. Remove the oil bath and leave the reaction mixture to cool down to room temperature under inert atmosphere.
▲ CRITICAL STEP Once the solution has sufficiently cooled down, perform the workup without delay. Ammonium formate gives ammonia and formic acid in the reaction medium. Postponing the workup allows formylation of the tetramer and hexamer amine to take place (**Fig. 11b**).
9. Dilute the reaction mixture with 50 ml of CH_2Cl_2. Filter the mixture through a 125-ml sintered glass filter funnel (porosity grade 3) filled with a short pad of packed Celite. Rinse the Celite with 50 ml of CH_2Cl_2 to remove all yellow product.
10. Remove the solvent using a rotary evaporator.
11. Dissolve the residue in 250 ml of CH_2Cl_2 and add 200 ml of water. Transfer the mixture to a 1-liter separation funnel and separate the layers. Wash the organic layer with 200 ml of distilled water and 200 ml of saturated aqueous NaCl. Dry the organic layer over $MgSO_4$ for 1 h. Filter the mixture through a 125-ml sintered glass filter funnel (porosity grade 3). Rinse the residue three times, each with 5 ml of CH_2Cl_2. Remove the solvent using a rotary evaporator. Add 150 ml of dry toluene into the flask to remove residual water as an azeotrope using a rotary evaporator. Repeat this treatment once. Dry the residue under vacuum for 5 h. The bright yellow tetramer amine **10** and hexamer amine **12** (typical yield 97% for tetramer amine **10**, i.e., 4.90 g and typical yield 96% for the hexamer amine **12**, i.e., 6.60 g) are used without further purification in the coupling reactions (Steps 25–37 of the PROCEDURE). For analytical data, see **Supplementary Figure 10** (tetramer amine **10**), **Supplementary Figure 12** (hexamer amine **12**) and ref. 38. Compounds **10** and **12** can be stored at room temperature and in the dark during at least 3 months.
▲ CRITICAL STEP The amine should be thoroughly dried, as the coupling reaction is moisture

● TIMING
Preparation of tetramer amine 10 and hexamer amine 12: 24.5 h
Steps 1–5: 1.5 h
Step 6: 12 h
Steps 7–11: 11 h

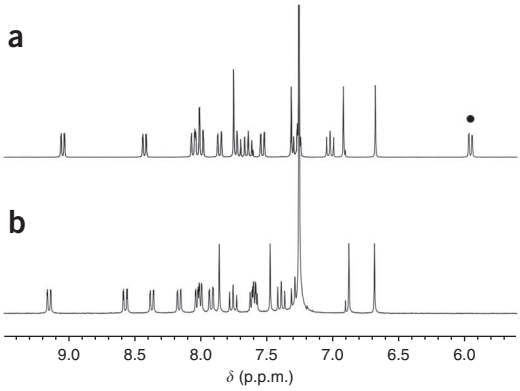

Figure 7 | Preparation of the tetramer amine. (**a**,**b**) Part of the ^{1}H-NMR spectra (25 °C, $CDCl_3$, 300 MHz) of tetramer amine **10** (**a**) and tetramer **9** (**b**). The characteristic peak is marked with a dot.

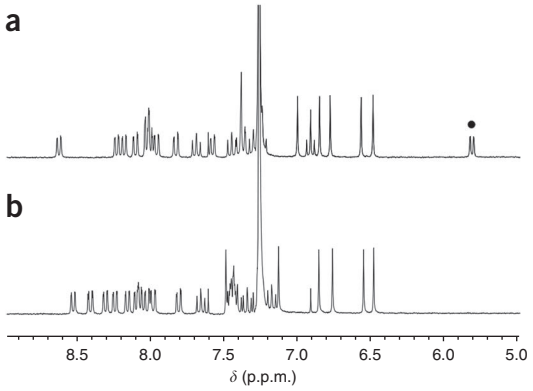

Figure 8 | Preparation of the hexamer amine. (**a**,**b**) Part of the ^{1}H-NMR spectra (25 °C, $CDCl_3$, 300 MHz) of hexamer amine **12** (**a**) and hexamer **11** (**b**). The characteristic peak is marked with a dot.

30| By using a dry syringe, deliver dry DIPEA into the reaction flask and cool the solution to 0 °C by placing the reaction flask in an ice bath (solution A).

Amine starting material	Volume of dry DIPEA (ml)	Amount of dry DIPEA (mmol)
Monomer amine **3**	21.3	122
Dimer amine **7**	5.0	28.7
Tetramer amine **10**	4.7	27.0
Hexamer amine **12**	4.3	24.7

31| By using a dry syringe, deliver dry CH_2Cl_2 to dissolve the appropriate amount of thoroughly dried acid chloride in the two-necked round-bottomed flask in which it was prepared (solution B).

Amine starting material	Acid chloride	Volume of CH_2Cl_2 (ml)
Monomer amine **3**	**4** (monomer)	50
Dimer amine **7**	**8** (dimer)	40
Tetramer amine **10**	**8** (dimer)	40
Hexamer amine **12**	**8** (dimer)	40

32| Transfer the acid chloride solution (solution B) slowly through a dry cannula (or syringe) through the septum to the cold amine solution (solution A). Rinse the flask of solution B three times, each with 5 ml of dry CH_2Cl_2.

33| Stir the reaction mixture further for 12 h at room temperature.

34| Ensure complete amine conversion by ^{1}H NMR spectroscopy before proceeding. By using a dry syringe, take out a 0.05-ml aliquot of the reaction solution and place it into an NMR tube. Remove the solvent using a high-vacuum line. Dissolve the residue in 0.5 ml of $CDCl_3$ and record a ^{1}H NMR spectrum. When no amine is present, continue to the next step. If some amine is still present, as judged by the characteristic proton resonance in the ^{1}H NMR spectrum, add freshly prepared acid chloride solution (1.1 equiv. to the amount amine of derived from of the ^{1}H NMR spectrum) and continue stirring at room temperature.

Amine starting material	Proton resonance (p.p.m.)
Monomer amine **3** (**Fig. 3**)	5.11
Dimer amine **7** (**Fig. 5**)	5.55
Tetramer amine **10** (**Fig. 7**)	6.00
Hexamer amine **6** (**Fig. 8**)	5.85

35| Transfer the reaction mixture to a 1-liter separation funnel and add CH_2Cl_2. Wash the organic layer consecutively with equal volumes of water, saturated aqueous $NaHCO_3$, 0.1 M aqueous HCl and saturated aqueous NaCl. Dry the organic layer over $MgSO_4$ for 1 h. Filter the mixture through a 125-ml sintered glass filter funnel (porosity grade 3). Remove the solvent using a rotary evaporator.

Synthesis of:	Volume of CH_2Cl_2 (ml)	Volume of each other wash (ml)
Dimer **5**	50	200
Tetramer **9**	120	200
Hexamer **11**	100	150
Octamer **13**	60	200

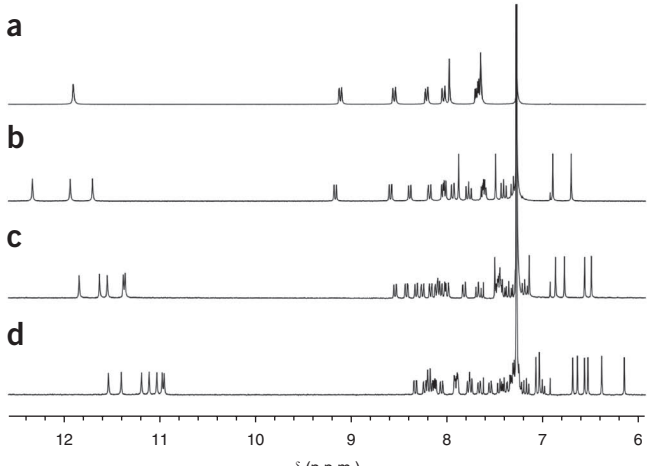

Figure 9 | Products of coupling reactions. (**a**–**d**) ^{1}H-NMR spectrum (25 °C, $CDCl_3$, 300 MHz) of dimer **5** (**a**), tetramer **9** (**b**), hexamer **11** (**c**) and octamer **13** (**d**).

36| Triturate the crude residue with warm methanol (50 °C); isolate the precipitate by filtration through a 125-ml sintered glass filter funnel (porosity grade 3) and wash it three times, with 50 ml of methanol each time.

Synthesis of:	Volume of warm methanol (ml)
Dimer **5**	150
Tetramer **9**	100
Hexamer **11**	100
Octamer **13**	100

37| Dry the yellow solid under vacuum for 12 h. For analytical data, see **Figure 9**, **Supplementary Figure 13** and ref. 38. For a crystal structure of **13**, see the **Supplementary Data** and ref. 6. For a full 2D NMR investigation of **13**, including the full assignment of its ^{1}H and ^{13}C resonances, see ref. 43.

Synthesis of:	Yield (range %)	Maximum mass (g)
Dimer **5**	89–97	11.8
Tetramer **9**	88–98	5.3
Hexamer **11**	90–98	7.0
Octamer **13**	90–98	8.4

■ **PAUSE POINT** Compounds **5**, **9**, **11** and **13** can be stored at room temperature for at least 1 year.
? TROUBLESHOOTING

? TROUBLESHOOTING
Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting table.

Step	Problem	Possible reason	Solution
Box 1 (step 4)	Intermediates are observed in the control ^{1}H NMR of monomer **1** and dimer **5** reduction	Incomplete nitro group reduction because of too short reaction time, too weak stirring or insufficient hydrogen pressure	Stir the reaction mixture long enough and vigorously under a sufficient hydrogen atmosphere If the problem is identified by the control ^{1}H NMR, simply resume the reaction by applying a freshly filled hydrogen balloon and continue stirring If the problem is identified after workup, restart the reduction of the crude mixture as described

(continued)

TABLE 1 | Troubleshooting table (conitnued).

Step	Problem	Possible reason	Solution
Box 4 (step 7)	Side products are observed during and after reducing tetramer **9** and hexamer **11**	Intermediates are observed because of incomplete nitro group reduction	When encountered after 12 h, add more ammonium formate solution and continue stirring at 95 °C and monitor the reaction by ^{1}H NMR spectroscopy
		Side reactions occurred because of postponing the workup	Perform the workup immediately after the reaction mixture has sufficiently cooled down If the side reactions cannot be avoided, purify the crude mixture by column chromatography using CH_2Cl_2/cyclohexane (5:1 vol/vol) as eluent
37	Low coupling yields or incomplete amine conversion	Traces of water are present in the reaction mixture	Dry the starting compounds thoroughly (amines, acids and acid chlorides) Use well-dried solvents and reagents Prevent moisture from entering the reaction flask After 12 h, when amine is still observed, add new freshly prepared acid chloride to the reaction mixture and continue stirring at room temperature
	Impurities are observed after a coupling step, making purification by recrystallization difficult and low yielding	The protocol was not rigorously followed and the coupling step was carried out using an impure amine or an acid chloride that contained residual oxalyl chloride	Chromatographic purification on silica gel is possible but difficult and costly on a large scale It is indispensable before proceeding to the next step Depending on the nature of the impurity being removed, different solvent mixtures may be recommended as the mobile phase (such as CH_2Cl_2/cyclohexane, CH_2Cl_2/methanol, toluene/ethyl acetate)

● TIMING

Preparation of monomer 1: 3.5 d

Steps 1–7, preparation of dimethyl 2-(2-nitrophenylamino)-fumarate (**14**): 42 h (Steps 1 and 2: 30 min; Steps 3 and 4: 1 d; Step 5: 30 min; Step 6: 12 h; Step 7: 5 h)

Steps 8–15, preparation of methyl-8-nitro-(1H)-4-quinolinone-2-carboxylate (**15**): 8 h 45 min (Steps 8–10: 1 h; Step 11: max. 15 min; Step 12: 1.5 h; Steps 13 and 14; 1 h; Step 15: 5 h)

Steps 16–24, synthesis of methyl-4-isobutoxy-8-nitroquinoline-2-carboxylate (**1**): 32.5 h (Steps 16–19: 2.5 h; Step 20: 12.5 h; Steps 21 and 22: 12 h; Steps 23 and 24: 5.5 h)

Preparation of dimer 5: 5–6 d

Box 1 (steps 1–5), preparation of monomer amine **3**: 19 h

Box 2 (steps 1–16), preparation of monomer acid chloride **4**: 44 h

Steps 26–37, coupling reaction: 29.5 h

Preparation of tetramer 9: 5–6 d

Box 1 (steps 1–5), preparation of dimer amine **7**: 19 h

Box 3 (steps 1–16), preparation of dimer acid chloride **8**: 28 h

Steps 26–37, coupling reaction: 29.5 h

Preparation of hexamer 11: 5–6 d

Box 3 (steps 1–16), preparation of dimer acid chloride **8**: 28 h

Box 4 (steps 1–9), preparation of tetramer amine **10**: 24.5 h

Steps 26–37, coupling reaction: 29.5 h

Preparation of octamer 13: 5–6 d
Box 3 (steps 1–16), preparation of dimer acid chloride **8**: 28 h
Box 4 (steps 1–5), preparation of hexamer amine **12**: 24.5 h
Steps 26–37, coupling reaction: 29.5 h

ANTICIPATED RESULTS

The synthesis described here allows the conversion of 16 g of methyl-8-nitro-(1H)-4-quinolinone-2-carboxylate **1** into 8.4 g of octamer **13** in 17 reaction steps, without any chromatographic purification. The overall yield over 19 steps from commercially available 2-nitroaniline ranges from 23 to 33%. This synthesis requires high-yielding coupling reactions between amines and acid chlorides, which in turn require high purity of dimer, tetramer and hexamer amines, as well as high purity of monomer and dimer acid chlorides. Purity can be easily determined by ^{1}H NMR spectra.

A typical yield of a correctly performed coupling reaction is around 90%. The resulting crude mixtures do not require column chromatography purification, but they are purified by crystallization or precipitation. Monitoring a coupling reaction by ^{1}H NMR spectroscopy clearly indicates complete amine conversion and may allow substantial reduction of the reaction time from the prescribed 12 h. The ^{1}H NMR spectra of the oligomers show sharp signals spread over a large range of chemical shifts. Amide protons are well defined between 10 and 12.5 p.p.m. and give a clear indication of product purity. A comparison of the dimer, tetramer, hexamer and octamer spectra shows an upfield signal shift because of increasing intramolecular ring-current effects as strand length increases (**Fig. 9**).

The yields drop markedly when starting materials are impure and coupling reactions are poorly implemented, which in turn makes purification very complicated. A moisture-free reaction medium during coupling is an obvious requirement: acid chloride and amine should be thoroughly dried, and dry solvents and base should be used. When water is present, the acid chloride will be hydrolyzed and the amine will not be completely consumed after 12 h. Excess acid can be easily removed, but the amine is difficult to remove even via column chromatography because of similar polarity with the product (**Fig. 10**). Adding new freshly prepared acid chloride, to ensure complete amine conversion, is an efficient way to avoid difficult chromatography.

Although the reduction of the nitro compounds described in **Boxes 3** and **4** are quantitative, intermediates and side products might be observed. Incomplete reduction of the nitro group or postponing the workup gives crude mixtures, which are difficult to purify and make the next reactions complicated (**Fig. 11**).

ANALYTICAL DATA

Octamer **13**
Yellow solid
Melting point: >250 °C

^{1}H NMR (300 MHz, $CDCl_3$): δ 11.51 (1H, s), 11.38 (1H, s), 11.17 (1H, s), 11.09 (1H, s), 11.01 (1H, s), 10.95 (1H, s), 10.93 (1H, s), 8.32 (1H, dd, J = 1.3, 6.7 Hz), 8.21 (1H, dd, J = 1.3, 6.7 Hz), 8.18 (1H, br), 8.16 (1H, s), 8.11 (2H, td, J = 6.7, 1.3 Hz), 8.03 (1H, dd, J = 1.3, 6.7 Hz), 7.89 (3H, m), 7.74 (2H, td, J = 7.4, 1.3 Hz), 7.65 (1H, dd, J = 1.2, 7.7 Hz), 7.53 (1H, dd, J = 1.2, 7.6 Hz), 7.46 (2H, m), 7.29 (6H, m), 7.15 (1H, m), 7.06 (1H, s), 7.02 (1H, s), 6.99 (1H, t, J = 8.0 Hz), 6.67 (1H, s), 6.62 (1H, s), 6.55 (1H, s), 6.51 (1H, s), 6.37 (1H, s), 6.13 (1H, s), 3.90 (13H, m), 3.67 (3H, m), 3.00 (3H, s), 2.52 (2H, m), 2.35 (5H, m), 2.19 (1H, m), 1.36 (12H, m), 1.20 (36H, m).

^{13}C NMR (75 MHz, $CDCl_3$): δ 163.88 (C), 162.97 (C), 162.91 (C), 162.88 (C), 162.79 (C), 162.77 (C), 162.57 (C), 162.48 (C), 162.03 (C), 161.17 (C), 160.93 (C), 160.69 (C), 160.20 (C), 159.88 (C), 159.43 (C), 159.41 (C), 153.17 (C), 150.05 (C),

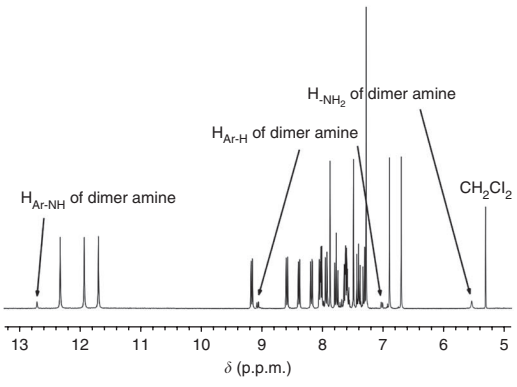

Figure 10 | Crude ^{1}H-NMR spectrum (25 °C, $CDCl_3$, 300 MHz) of tetramer **9** showing traces of dimer amine **7**.

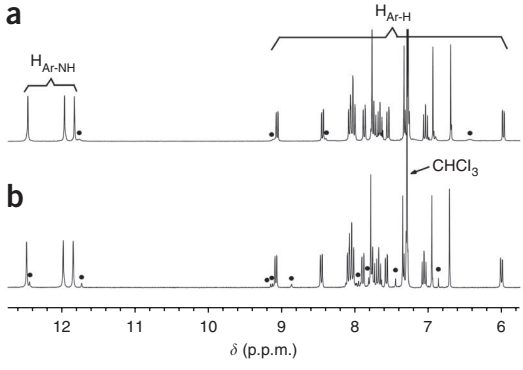

Figure 11 | Part of the ^{1}H-NMR spectra (25 °C, $CDCl_3$, 300 MHz) showing the amide and aromatic proton resonances of tetramer amine **10**. (**a**,**b**) Side products due to incomplete reduction (**a**) or undesired amine formylation by postponing the workup (**b**) are marked with dots.

149.32 (C), 149.25 (C), 148.97 (C), 148.83 (C), 148.26 (C), 145.11 (C), 144.80 (C), 138.81 (C), 138.77 (C), 138.23 (C), 138.12 (C), 137.87 (C), 137.72 (C), 137.68 (C), 137.49 (C), 134.25 (C), 133.72 (C), 133.35 (C), 132.96 (C), 132.82 (C), 128.01 (CH), 127.67 (CH), 127.04 (CH), 126.92 (CH), 126.41 (CH), 125.91 (CH), 125.89 (CH), 125.79 (CH), 125.65 (CH), 123.88 (CH), 123.65 (C), 122.71 (C), 122.49 (C), 122.41 (C), 121.70 (C), 121.53 (C), 121.40 (C), 117.41 (CH), 117.14 (CH), 117.06 (CH), 116.92 (CH), 116.81 (CH), 116.78 (CH), 116.45 (CH), 116.34 (CH), 116.17 (CH), 116.15 (CH), 116.10 (CH), 116.02 (CH), 115.96 (CH), 115.62 (CH), 100.09 (CH), 99.94 (CH), 99.66 (CH), 99.29 (CH), 98.78 (CH), 97.98 (CH), 97.76 (CH), 97.57 (CH), 75.63 (CH_2), 75.48 (CH_2), 75.45 (CH_2), 75.39 (CH_2), 75.36 (CH_2), 75.32 (CH_2), 75.14 (CH_2), 74.83 (CH_2), 52.09 (CH_3), 28.36 (CH), 28.33 (CH), 28.30 (CH), 28.28 (CH), 28.24 (CH), 28.21 (CH), 28.18 (CH), 28.08 (CH), 19.75 (CH_3), 19.72 (CH_3), 19.69 (CH_3), 19.65 (CH_3), 19.62 (CH_3), 19.60 (CH_3), 19.53 (CH_3), 19.51 (CH_3), 19.46 (CH_3), 19.43 (CH_3), 19.40 (CH_3), 19.37 (CH_3), 19.31 (CH_3).

IR (KBr) ν, (cm^{-1}): 2,960, 1,684, 1,539, 1,469, 1,419, 1,357, 1,331, 1,264, 1,212, 1,114, 1,053, 878, 817, 759.

TOF-MS *m/z*: 1,999.73 $[M+H]^+$, 2,021.73 $[M+Na]^+$, 2,037.70 $[M+K]^+$.

Note: Supplementary information is available in the online version of the paper.

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