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# Synthesis of polyamino nitriles, en route to acylpolyamine neurotoxins, via the regioselective michael cyanoethylation of unprotected polyamines. Unusual behaviour of 1-(2-aminoethyl)piperazine

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ABSTRACT

The Michael cyanoethylation of 1-(2-aminoethyl)piperazine, 4,4′-methylenebis(cyclohexylamine) and bis(3-aminopropylamine)amine, leading to acrylonitrile free (<100 ppm) polyamino nitriles, as a key step in the synthesis of higher polyamines useful in the synthesis of acylpolyamine neurotoxins, was carried out regioselectively on a multigram scale by careful tuning of reaction conditions, without a necessity to protect nitrogen atoms. The higher reactivity of primary amino groups in aliphatic diamines and triamines [as in bis(3-aminopropylamine)amine] was also observed in the cyclic amine, 4,4′-methylenebis(cyclohexylamine), but reversed in 1-(2-aminoethyl)piperazine. The compounds with a dicyanoethylated nitrogen atom were thermally less stable than the monocyanoethylated ones.



## 1. Introduction

Polyamines, both synthetic and naturally occurring, are an important class of biologically active compounds, for example, putrescine, cadaverine, spermidine or spermine, which were first described in XVII century.[1] Their analogues and conjugates to other biomolecules in a linear or cyclic fashion have been synthesized and recently reviewed by Karigiannis and Papaioannou.[2] They are broadly applicable for drug delivery, therapeutics, and engineering nanomaterials. Polyamines, especially aliphatic ones, are also excellent hardeners for epoxy resins.[3] Higher polyamines of similar utility may be obtained by cyanoethylation of mono-, di- or polyamines with acrylonitrile followed by a reduction of the resulting nitriles to the corresponding amines. The synthetic problem is often in regioselectivity of this transformation. Therefore, protection of the relevant nitrogen atoms is required in such cases. An example of monocyanoethylation of primary alkylamines was reported by Short et al.[4] who used the protocol of Tarbell et al.[5] for the preparation of *N*-heptyl-1,3-diaminopropane from *N*-heptylamine.

Dicyanoethylation of primary nitrogen atoms in *N*-unprotected alkyl diamines (propane-1,3-diamine) and doubly $N^2$,$N^3$-Boc protected alkyl tetraamines [bis(3-aminopropyl)propane-1,3-diamine] followed by a reduction of the resulting nitriles to the corresponding tetra- and hexamines, was carried out by Threadgrill et al.[6] in the synthesis of polyamine–poly(ethyleneglycol) constructs for DNA binding and gene therapy. Monocyanoethylation of the secondary amine nitrogen atom in bis-(3-aminopropyl)amine followed by hydrogenation to give the relevant pentamine derivative was possible after a double *N*-Boc protection of both terminal primary nitrogen atoms of the starting amine.[6] When unprotected, the nitrogen atoms underwent a selective dicyanoethylation to give the dinitrile, which was reduced without isolation to the pentamine.[7] The sequence of dicyanoethylation of butane-1,3-diamine and reduction was utilized by Simons et al.[8] in a solid-phase synthesis of polyamine amino acid derivatives as HIV-1 Tat-TAR binding inhibitors and by Fusetani et al.[9] in a total synthesis of sinulamide, a tetraprenylated spermine derivative.

Hexane-1,6-diamine,[10] heptane-1,7-diamine,[11] octane-1,8-diamine[12] behaved in the same way as the lower analogues. A mono- and symmetrical dicyanoethylation/reduction concept was further developed by Israel et al.[13] in the synthesis of a series of linear aliphatic triamines and tetraamines containing from 2 through 12 methylene units as homologues of naturally occurring spermine and spermidine.

Other examples of mono- and dicyanoethylation of penta-,[14,15] hexa-[16] and heptamethylene[17] diamines and monocyanoethylation

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of the triamine [bis-(2-aminoethyl)amine][18] show that in acyclic polyamines primary nitrogen atoms are more reactive than the secondary ones.

In this paper, we show that the reverse reactivity is also possible with the piperazine derivative **1**, containing *N*-(2-aminoethyl) chain, and that selectivity in the Michael cyanoethylation of secondary nitrogen atoms in aliphatic polyamines can also be achieved without *N*-protection. Thus, in the syntheses of higher polyamines two synthetic operations (protection and deprotection) may be avoided.

Our interest in polyamines is manifold and connected with their potential as building blocks in the synthesis of modified acylpolyamine neurotoxins, for instance **I**, NPTX-594, isolated from the venom of a Madagascar Joro spider, *Nephila madagascariensis*[19] and **II**, philanthotoxin-433 [PhTX-433], an active constituent of the venom of the Egyptian digger wasp *Philanthus triangulum*,[2] which is a nonselective inhibitor of ionotropic glutamate receptors (iGluRs) and nicotinic acetylcholine receptors (nAChRs).[20] The bioactivity of the latter suppresses neurotransmission in mammalian brain, which is extremely important in the treatment of neurological dysfunctions, including Alzheimer's disease.

A recent contribution of Franzyk et al.[21] on the synthesis of a novel type of philanthotoxins, for instance **III**, containing piperazine and piperidine building blocks with conformational rigidity, increased lipophilicity and altered proteolytic properties shows that investigations in this field are directed towards modifications of properties and biological activity by alteration of the polyamine chain of these neurotoxins (Fig. 1).

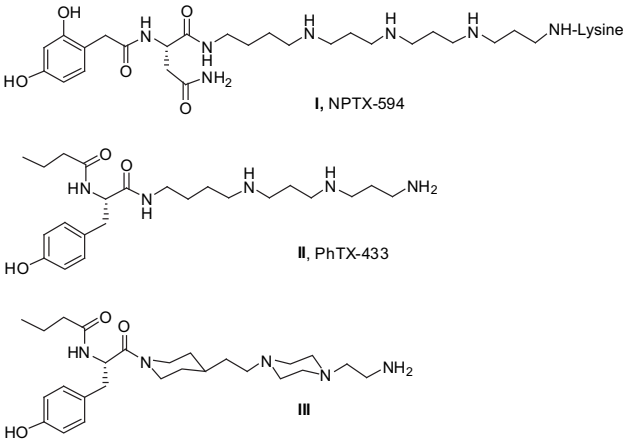


**Figure 1.** Natural and modified acylpolyamine neurotoxins.

A separate problem, which we solved, was elaboration of experimental and HPLC analytical procedures for a removal of toxic acrylonitrile below the accepted level of 100 ppm (usually 75–85 ppm), which we used as a reagent for the Michael cyanoethylation of polyamines. Acrylonitrile is classified as a recognized human carcinogen. Overexposure can cause eye irritation, nausea, vomiting, headache, sneezing, weakness, and lightheadedness. At high concentrations, the effects of exposure may go on to loss of consciousness and death.[22]

In syntheses of polynitriles, acrylonitrile has to be used in some procedures in a large excess (see Experimental) and then its excess has to be removed. However, the evaporation is not sufficient and crude products still contain certain amounts of this toxic substance, which should be removed on earlier stages of synthesis of acylpolyamine neurotoxins.

## 2. Results

In these investigations, three different amines: 1-(2-aminoethyl)piperazine **1**, 4,4′-methylenebis (cyclohexylamine) **2** and bis(3-aminopropylamine)amine **3** have been chosen to produce several higher polyamines, free of toxic acrylonitrile, as candidates for modification of the polyamine chain of the neurotoxins (Fig. 2).

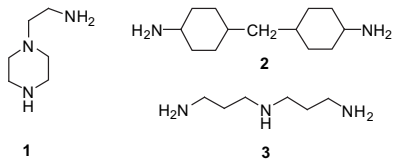


**Figure 2.** Representative amines used in the regioselective Michael cyanoethylation.

### 2.1. Cyanoethylation of 1-(2-aminoethyl)piperazine 1

This structurally interesting amine posseses one primary aliphatic, one secondary and one tertiary ring nitrogen atom. It is also an example of amine containing a six-membered ring and aliphatic side chain and, as other unprotected aliphatic amines, it should preferentially react at the primary chain nitrogen atom with 1 equiv of acrylonitrile to give 3-(2-piperazin-1-yl-ethylamino)-propionitrile **4**. Its reaction with the second equivalent of acrylonitrile, should, due to the presence of two secondary amino moieties, produce 3-{4-[2-(2-cyanoethylamino)-ethyl]-piperazin-1-yl}propionitrile **5** and/or 3-[(2-cyanoethyl)-(2-piperazin-1-yl-ethyl)-amino]-propionitrile **6** (Fig. 3). Excess acrylonitrile should obviously lead to 3-(4-{2-[bis-(2-cyanoethyl)-amino]-ethyl}-piperazin-1-yl)-propionitrile **8**. In our experiments on the Michael addition reaction of **1** to acrylonitrile, it was unexpectedly found that the secondary amine group in **1** was more reactive than the primary one, and only compounds **5**, **7**, **8** could be selectively obtained (Fig. 3, Table 1).

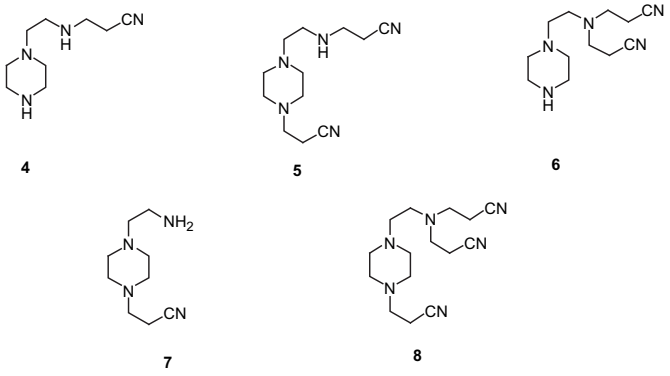


**Figure 3.** All possible products **4**–**8** and selectively obtained ones **5**, **7**, **8** in the Michael addition of **1** to acrylonitrile.

We were successful in the isolation of all the three products in good and very good yields: **5** (88%), **7** (74%), and **8** (94%) after optimization of the reaction conditions[23,24] (Table 1 and Experimental). Compounds **4** and **6** have never been observed in the reaction mixtures. Moreover, it was not possible to reverse the observed reactivity of the ring NH and chain $NH_2$ nitrogen atoms by varying stoichiometry, temperature, solvents or acidic activating/deactivating additives including the BMIM·$PF_6$ ionic liquid (1-butyl-3-methylimidazolium hexafluoro-phosphate). Other attempts were also made to obtain mono- and bisaddition products **4** or **6**. Thus, an attempt at the thermal mono- or bis-decyanoethylation was made using the trisaddition product **8** as a substrate at 200 °C/4 h, under reduced

**Table 1**
Optimization of reaction conditions of the Michael addition reaction of **1** to acrylonitrile (entries 1–12) and thermal decomposition of **8** (entries 13, 14)

| No | Solvent | Amount of acrylonitrile (equiv) | Additive | Conditions | Results |
|---|---|---|---|---|---|
| 1 | MeOH | 1.0 | — | 0 °C 3 h, rt 20 h | **1**:**7**:**5**=13:74:13 |
| 2 | MeOH | 2.1 | — | 0 °C 3 h, rt 18 h | **5** (~95%) |
| 3 | Benzene | 1.05 | — | 65–70 °C, 5 h | **1**:**7**:**5**=7:83:11 |
| 4 | Benzene | 2.1 | — | 65–70 °C, 5 h | **7**:**5**=60:40 |
| 5 | Benzene | 3.15 | — | 65–70 °C, 5 h | **7**:**5**=30:70 |
| 6 | MeOH | 1.05 | AcOH (1 eqiuv) | 0 °C, 3 h; rt, 17 h | Cyanoethylation at the piperazine nitrogen |
| 7 | *i*-PrOH | 1.05 | — | 0 °C, 2 h, rt, 20 h | A mixture of **1**, **7** (Main product), **5** and a small amount of unidentified compound |
| 8 | Toluene | 1.05 | Silicagel (0.6 g per 0.5 g of amine | rt, 20 h | **7** (Main product), a small amount of **5** |
| 9 | THF | 1.05 | — | rt, 20 h | **7** (Main product), a small amount of **1** and **5** |
| 10 | $CH_2Cl_2$ | 1.05 | — | rt, 20 h | **7** (Main product), a small amount of **1** and **5** |
| 11 | Without solvent | 1.05 | BMIM·$PF_6$ (60 mg per 0.5 g of amine) | rt, 20 h | **7** (Main product), a small amount of **1** and **5** |
| 12 | MeOH | 10 | — | rt, 5 days | >95% of **8** |
| 13 | — | — | — | 110 °C, 3 h, 1 mmHg | No decomposition (>95% of **8**) |
| 14 | — | — | — | 140 °C, 5 h, 4 mmHg | No decomposition (>95% of **8**) |

pressure to remove the releasing acrylonitrile. It revealed a high thermal stability of this compound since only traces of decomposition products accompanying the substrate were observed. In another experiment, **8** was heated at 100 °C under reduced pressure for 6 h with a catalytic amount of solid NaOH hoping for the β-elimination of acrylonitrile. In this case, however, a complex mixture of products was obtained.

## 2.2. Cyanoethylation of 4,4′-methylenebis(cyclohexylamine) 2

In this reaction, which was carried out with stoichiometry of acrylonitrile/**2**=2:1, according to the protocol described in Experimental, the expected disubstituted product **10** was obtained almost quantitatively as a white solid with mp=49–52 °C (Fig. 4).

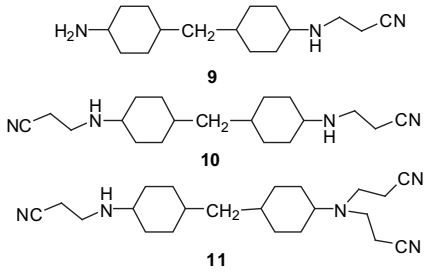


**Figure 4.** Products of the Michael addition reaction of **2** to acrylonitrile.

The starting 4,4′-methylenebis(cyclohexylamine) **2** was used as a mixture of *trans–trans*, *trans–cis* and *cis–cis* isomers in a ratio 50:40:10, respectively, and the product **10** was also isolated as a mixture of the corresponding isomers in a similar ratio. In order to facilitate unambiguous $^1$H- and $^{13}$C NMR spectroscopic analysis of these complex reaction mixtures, the monoaddition **9** and the trisaddition products **11** were additionally synthesized using varying amounts of acrylonitrile. Although their isolation in a pure state by a simple column chromatography was not possible, the relevant chromatography fractions containing varying amounts of **9** and **11** could be analyzed with the CIMS technique and the obtained results were very helpful in drawing final conclusions. First, the reaction leading to **10** from **2** was carried out on a 1 g scale in methanol. Then, the reaction was scaled up to 70 g, 100 g and finally to 180 g of **2**, increasing the necessary reaction time to 2.5 h, 3.5 h and 5 h and the volume of MeOH, used as a solvent, to 140, 200 and 300 mL, respectively.

Due to a beneficial thermal effect during addition of acrylonitrile, the temperature of the reactions carried out on larger scales was maintained at a slightly higher level of 30–35 °C than in the original small scale procedure to keep the mixture liquid. The final product **10** contained only 75 ppm of acrylonitrile, determined by the HPLC analysis using an optimized mixture of methanol/water containing a minimum of 55 v/v parts of methanol and 45 v/v parts of water.

## 2.3. Bis(3-aminopropylamine)amine 3

*2.3.1. Synthesis of **12** versus **13**.* In case of the amine **3**, the selectivity of the Michael cyanoethylation could again be achieved by tuning the reaction conditions. Thus, synthesis of the symmetrical trisaddition compound **12** possessing two unprotected secondary amino groups was carried out with a 20-fold excess of acrylonitrile in MeOH (Fig. 5). This reaction was successful only when terminated before another symmetrical pentakisaddition product **13** was formed. We were lucky to find such selective reaction conditions in which the reaction temperature was lowered to 0 °C and the reaction time was

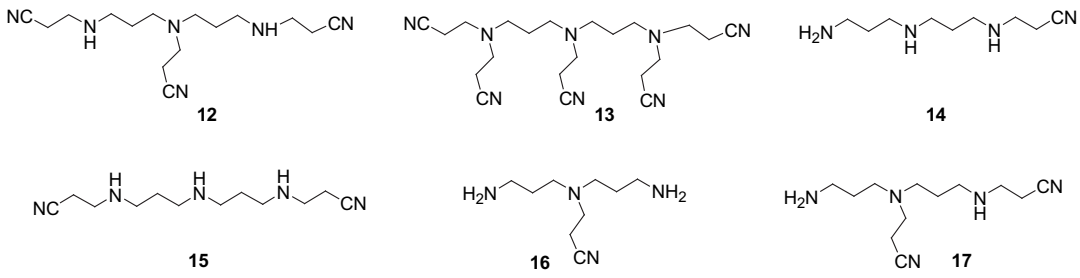


**Figure 5.** Temperature and time dependent products of the selective Michael cyanoethylation reaction of **3**.

shortened to 30 min. The compound **13** was also synthesized as the exclusive reaction product using the same excess of 20 equiv of acrylonitrile, but at higher temperatures (room temperature) within a longer reaction time (5 days). Thus, in the presence of excess acrylonitrile, the following order of reactivity of the nitrogen atoms in this amine was found. First, the two terminal primary and the middle secondary nitrogen atoms easily underwent cyanoethylation to give selectively **12** at 0 °C. Then, the reaction stopped and two remaining secondary nitrogen atoms could be slowly cyanoethylated at room temperature to afford **13**. The formation of compounds **12** and **13** was confirmed based on the mass balance, FAB, $^{15}$N NMR both INVGATE (with integration) and INEPT, $^{13}$C NMR and TLC. Especially, the INVGATE experiments were very helpful in a quick differentiation of both compounds based on the number of nitrile groups bound to different nitrogen atoms (2/1 in **12** vs 4/1 in **13**). The trisaddition product **12** was a liquid soluble in water in any proportion in contrast to the pentakisaddition product **13**, which was an oil insoluble in water. The reaction was scaled up and 500 g of the desired product **12** could be synthesized under laboratory conditions in five runs. According to the HPLC analysis, using a reverse phase column and water as an eluent, this product contained 85 ppm (i.e., <the threshold 100 ppm, vide infra) of acrylonitrile.

### 2.4. Inadequacy of the CIMS technique for analysis of cyanoethylated products

In the experiment with 20 equiv of acrylonitrile at room temperature in which **13** was formed, first spectral data indicated the formation of the trisaddition product **12** based on CIMS spectrum (M+1=291) and $^{13}$C NMR spectrum in which signals characteristic for a symmetrical compound (for instance **12**) were observed. However, further INVGATE $^{13}$C NMR experiments, mass balance of the reaction and MS-FAB (M+1=397) showed that the pentakisaddition product **13** was formed directly. An important conclusion was that the FAB but not the CI was the appropriate technique for analysis of the cyanoethylated products. Apparently, the thermal decomposition of **13** was intensively occurring in the spectrometer chamber during the CI experiment before or during evaporation of this compound and therefore according to the stochastic curve, signals due to all possible decomposition products were observed in the spectrum, together with the main signal at $m/z$=291(M+1) due to **12**. A less intensive decomposition occurred in the FAB experiment showing the relevant parent peak as the main one.

### 2.5. Thermal stability of the cyanoethylated products of 3

*2.5.1. Thermal decomposition of **13**.* The results obtained in the MS experiments suggested that the thermal decomposition reaction of **13** might lead to a formation of products containing a lower number of cyanoethylated moieties. Thus, the pentakisaddition product **13** was mixed with the starting amine, used as a scavenger of acrylonitrile and heated to 160 °C without a solvent at atmospheric pressure. The TLC analysis and $^{13}$C NMR spectrum, however, indicated the formation of a complex mixture containing products **12**, **14**–**17** as well as other minor products of thermal decomposition. Thus, compound **12** was formed as a sum of two reactions: the decyanoethylation of **13** and the cyanoethylation of the added amine **3** as well as cyanoethylations of lower substituted products **14**–**17**. The latter could be formed only from the cyanoethylation of **3** since **12** in contrast to **13** was thermally stable and did not undergo easy decomposition. As a conclusion and a rule, compounds with dicyanoethylated nitrogen atom, i.e., **13** and **17** were thermally less stable than monocyanoethylated products (**12**, **14**–**16**).

### 2.6. Synthetic attempts with varying amounts of acrylonitrile and the amine 3

In contrast to results obtained with 20 equiv of acetonitrile, reactions of the amine **3** with acrylonitrile used in various amounts from 2 to 6 equiv gave complex reaction mixtures. For instance, the TLC analysis of the crude product of the reaction between 1 equiv of amine **3** and 4 equiv of acrylonitrile, revealed three well resolved spots. Column chromatography (silica gel, methanol+1% aq ammonia) of this product allowed separation of three corresponding fractions. The least polar fraction contained the compound **13**, the second fraction was a tetrakisaddition product and the third one contained the trisaddition compound **12**, as indicated by $^{13}$C NMR spectroscopy. The most polar compounds **3**, **14**–**17** remained on the starting line. The conclusion was that the Michael cyanoethylation of the amine **3** had to be carried out with a large excess of acrylonitrile (20 equiv) under the tuned reaction conditions. Otherwise, the reaction was not selective and more than one product was always formed.

### 2.7. Synthetic attempts to improve selectivity using other solvents and additives

In order to improve selectivity of the reactions, the following were employed: from 2 to 6 equiv of acrylonitrile per 1 equiv of **3**, various solvents, additives and temperature. In acetonitrile, homogenous reactions were slow and not selective at room temperature and the results were similar to those obtained in MeOH. In triethylamine and diethyl ether, the heterogenous reactions were even slower, however, the selectivity in the amine solution was slightly higher than in diethyl ether. The reaction of a complex of the amine **3** with copper (II) acetate, synthesized in dichloromethane to protect and decrease the reactivity of the secondary nitrogen atom in **3**, failed with 2 equiv of acrylonitrile. Only the compound **15**, having three secondary amino groups (which was not synthesized so far) was formed in 20% yield, within 2 days at room temperature.

## 3. Conclusions

In conclusion, the simplest method of synthesizing higher, biologically active polyamines is based on the two-step sequence involving the Michael cyanoethylation of lower polyamines/reduction of the resulting polynitriles. The key cyanoethylation step is sometimes complex in view of the starting amine and reactivity of nitrogen atoms of various characters. Protection of secondary nitrogen atoms in aliphatic polyamines is usually a solution to this problem but requires two additional steps.

In this paper, we investigated the reactivities of structurally different unprotected amines showing that the regioselectivity of the cyanoethylation may be achieved by careful tuning of reaction conditions (stoichiometry, reaction time and temperature) as for the triamine **3** and the obtained products **12** versus **13**. The usually higher reactivity of primary nitrogen atoms in aliphatic diamines, also observed in 4,4′-methylenebis(cyclohexylamine) **2** was reversed in the piperazine derivative **1** containing *N*-(2-aminoethyl) side chain. This allowed a selective synthesis of the products **5**, **7** and **8**, which, among others, may be utilized in synthesis of acylpolyamine neurotoxins. We also focused our attention on the quantitative removal of toxic acrylonitrile to the standard, safe level below 100 ppm. We removed it before the next reduction step of the nitrile groups, elaborating the individual experimental protocols and the HPLC analytical procedure for determination of the quantity of this toxic substance. The solution of this problem will be important in all cases when a polyamine is used in biological tests and transformations leading to biologically active products as well

as in other processes or final products having any contact with human being.

## 4. Experimental

### 4.1. General

$^1$H NMR (200 and 500 MHz) and $^{13}$C NMR (50 and 125 MHz) spectra were recorded using a Bruker AC-200 and a Bruker DRX-500 spectrometers, respectively. Mass spectra were obtained using a Finnigan Mat 95 spectrometer. Column chromatography was done using Merck silica gel (F254 60, 70–230 and 270–400 mesh). The Merck TLC plates were developed in methanol containing 1% aq ammonia for analysis of **12**–**17**. Starting amines and acrylonitrile were commercial products. Organic solvents were purified by standard procedures.

### 4.2. 3-{4-[2-(2-Cyanoethylamino)ethyl]-piperazin-1-yl}propionitrile (5)

To a stirred solution of the amine **1** (0.80 g, 6.2 mmol) in methanol (3 mL), acrylonitrile (0.66 g, 12.4 mmol) was added dropwise during 2 h at 0 °C. Stirring was continued for an additional 2 h at this temperature and for 20 h at room temperature. The solvent was evaporated and the crude product was purified by column chromatography using methanol–25% ammonium hydroxide in water (100:1) as an eluent. Yield 1.28 g (88%). Oil: $\nu_{max}$(film) 3309, 2943, 2816, 2247, 1461, 1352, 1161, 1012; $\delta_H$ (200 MHz, $CDCl_3$) 1.65 (1H, br s), 2.58–2.39 (14H, m), 2.75–2.61 (4H, m), 2.94 (2H, t, $J$=6.6 Hz); $\delta_C$ (50 MHz, $CDCl_3$) 118.6, 118.5, 57.1, 53.0, 52.6, 52.28, 45.3, 44.8, 18.4, 15.5; HRMS (EI): $M^+$, found 236.1882. $C_{12}H_{22}N_5$ requires 236.1875.

### 4.3. 3-[4-(2-Aminoethyl)piperazin-1-yl]propionitrile (7)

To a stirred solution of **1** (1.80 g, 13.9 mmol) in methanol (6 mL), acrylonitrile (0.74 g, 13.9 mmol) was added dropwise during 2 h at 0 °C. Stirring was continued for additional 2 h at this temperature and for 20 h at the room temperature. The solvent was evaporated, and the crude product was purified by column chromatography using methanol–25% ammonium hydroxide in water (100:1). Yield 1.88 g (74%).Oil: $\nu_{max}$(film) 3367, 2942, 2812, 2247, 1593, 1461, 1352, 1161, 1011; $\delta_H$ (200 MHz, $CDCl_3$) 1.28 (2H, br s), 2.58–2.31 (12H, m), 2.80–2.62 (4H, m); $\delta_C$ (50 MHz, $CDCl_3$) 117.9, 60.1, 53.7, 52.3, 51.6, 37.8, 14.7; HRMS (EI): $M^+$, found 183.1612. $C_9H_{19}N_4$: requires 183.1610.

### 4.4. 3-(4-{2-[Bis-(2-cyanoethyl)-amino]-ethyl}-piperazin-1-yl)-propionitrile (8)

To a stirred solution of the amine **1** (0.50 g, 3.87 mmol) in methanol (1.5 mL), acrylonitrile (2.16 g, 40.6 mmol) was added slowly at room temperature. Stirring was continued for 48 h, the solvent was evaporated, and the crude product was purified by column chromatography using methanol–25% ammonium hydroxide in water (100:1) as an eluent. Yield 0.85 g (94%). Oil: $\nu_{max}$(film) 3355, 3188, 2948, 2816, 2247, 1463, 1357, 1161, 1133, 1011; $\delta_H$ (200 MHz, $CDCl_3$) 2.58–2.35 (16H, m), 2.75–2.65 (4H, m),, 2.92 (4H, t, $J$=6.8 Hz); $\delta_C$ (50 MHz, $CDCl_3$) 118.6 (*C*N), 118.5 (2 *C*N), 56.4, 53.1 (2C), 53.0, 52.4 (2C), 50.7, 50.0 (2C), 16.9 (2*C*$H_2$CN), 15.6 (*C*$H_2$CN); HRMS (EI): $M^+$, found 288.2063. $C_{15}H_{24}N_6$ requires 288.2062.

### 4.5. 3-{4-[-(2-Cyanoethylamino)cyclohexyl-methyl]-cyclohexylamino}propionitrile (10)

4,4′-Methylenobis(cyclohexylamine) **2** (1.05 g, 5 mmol) was dissolved in methanol (2 mL) and to the resulting solution acrylonitrile (0.544 g, 10.25 mmol) was added dropwise with stirring during 2 hr at rt. Then, stirring was continued for additional 48 h and methanol was evaporated. The crude product was preliminarily kept under vacuum (1 hr, 25 °C, 1 mmHg) to give quantitatively 1.58 g of the crude product **10**. After the vacuum treatment, the amount of acrylonitrile still remained below 150–180 ppm. For the effective removal of acrylonitrile below 100 ppm, the crude product was further dissolved in methylene chloride and washed with water three times.

Another procedure involving azeotropic removal of acrylonitrile with EtOH was effective up to the level of 150 ppm. It involved dissolution of **10** in EtOH, evaporation of the azeotrope (twice, 1×200 mL, then 1×300 mL) and keeping the final product under vacuum for 10 h at 60–68 °C/0.01 Torr.

This reaction was scaled up (see the main text). The combined crude products from three runs (>500 g of **10**) after evaporation of methanol were dissolved in methylene chloride (2 L) and this solution was divided into two parts. Each volume was washed with water (3×500 mL) then dried over $MgSO_4$, filtered and evaporated to give **10** containing only 75 ppm of acrylonitrile.

White solid: mp=49–52 °C; [found: C, 72.12; H, 10.19; N, 17.62. $C_{19}H_{32}N_4$ requires C, 72.11; H, 10.18; N, 17.71]; $\nu_{max}$(KBr) 3461(br), 3312, 2921, 2847, 2246, 1650, 1448, 1130; $\delta_H$ (200 MHz, $CDCl_3$) 0.75–1.60 (m, 17H), 1.65–1.95 (5H, m), 2.32–2.44 (1H, m), 2.45–2.57 (4H, m), 2.66–2.78 (1H, m), 2.83–3.00 (4H, m); $\delta_C$ (50 MHz, $CDCl_3$) 118.64, 55.35, 52.92, 44.25, 42.28, 42.07, 34.10, 33.29, 32.40, 31.89, 29.31, 27.60, 19.02; $m/z$ (CI, isobutane) 317(100 $MH^+$); $m/z$ (FAB, +VE) 317(100 $MH^+$).

### 4.6. 3-(3-{(2-Cyanoethyl)-[3-(2-cyanoethylamino) propyl]amino}propylamino)propionitrile (12)

To a stirred solution of the amine **3** (46.0 g, 0.351 mol) in MeOH (175 mL), acrylonitrile (372 g, 7.02 mol, 462 mL) was added at 0 °C. The solution was stirred for 30 min at this temperature and an excess of acrylonitrile and MeOH was evaporated. To the remaining viscous liquid (103.7 g), EtOH (2×25 mL) was added, mixed and then evaporated to remove the azeotrope using water pump and then oil pump. The product contained not more than 5% of a tetrakisaddition compound (MS analysis), 2% of the starting amine **3** and 85 ppm of acrylonitrile. Oil: [found: C, 61.98; H, 8.83; N, 28.95. $C_{15}H_{26}N_6$ requires C, 62.04; H, 9.02; N, 28.94]; $R_f$ [MeOH+1% (25% $NH_3$ in water]=0.45; $\delta_H$ (200 MHz, $CDCl_3$) 2.86–2.93 (4H, m), 2.65–2.74 (6H, m), 2.44–2.54 (10H, m), 1.55–1.68 (4H, m); $\delta_C$ (50 MHz, $CDCl_3$) 118.4, 118.2, 50.2, 48.1, 47.8, 45.7, 43.8, 26.0, 17.2, 15,8; $\delta_{N15}$ (50 MHz, $MeNO_2$, INVGATE) 36.8 (2N), 38.2 (1N), 246.3 (1N), 246.4 (2N), $m/z$ (FAB, +VE) 291(100 $MH^+$).

### 4.7. 3-[{3-[{3-[Bis-(2-cyanoethyl)amino]propyl}-(2-cyanoethyl)amino]propyl}-(2-cyanoethyl)-amino]propionitrile (13)

A solution of the amine **3** (46.0 g, 0.351 mol) in acrylonitrile (372 g, 7.02 mol, 462 mL) was stirred for 5 days at room temperature and an excess of acrylonitrile was evaporated under reduced pressure to give quantitatively pure **13** as an oil insoluble in water: [found: C, 63.61; H, 7.92; N, 28.50. $C_{21}H_{32}N_{68}$ requires C, 63.61; H, 8.13; N, 28.26]; $\delta_H$ (200 MHz, $CDCl_3$) 1.53–1.69 (6H, m), 2.45–2.58 (14H, m), 2.61–2.76 (6H, m), 2.81–2.94 (6H, m); $\delta_C$ (50 MHz, $CDCl_3$, INVGATE) 15.8 (1C), 16.0 (4C), 24.3 (2C), 48.6 (5C), 50.3 (2C), 50.4 (2C), 118.6 (4C), 119.1 (1C); $m/z$ (FAB, +VE) 397 (100 $MH^+$).

### 4.8. Determination of a level of acrylonitrile. HPLC details

Concentrations of acrylonitrile in the synthesized products were determined by a high performance liquid chromatography. Measurements were carried out under specified conditions:

HPLC column:LKB reverse phase column, Lichrosorb RP18, 10 μm, 4×250 mm;
Mobile phase: water (the products obtained from **3**), water/methanol 45:55 (the product obtained from **2**);
Flow rate: 0.5 mL/min; Injection volume: 100 μL; Sample concentration: ca. 0.2 g/mL;
Detector UV wavelength: 195 nm; Retention time of acrylonitrile: 23 min (water as a mobile phase), 7 min (water/methanol 45:50 as a mobile phase).

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