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Mono- and bis-alkylation of glyoxal-bridged tetraazamacrocycles using mechanochemistry

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ABSTRACT: Glyoxal-bridged bisaminal tetraazamacrocyclic derivatives of 1,4,7,10-

tetraazacyclododecane (cyclen) and 1,4,8,11-tetraazacyclotetradecane (cyclam) can be N-functionalized to incorporate coordinating groups or for conjugation to biomolecules. Herein, we present an improved N-functionalization methodology using mechanochemistry which reduces reaction times in comparison with conventional synthetic routes. A range of six alkyl halides were reacted with cyclen and cyclam bisaminal derivatives in various ratios to form mono- and bis-functionalized quaternary ammonium salts. Cross-bridged cyclam, a key intermediate for CB-TE2A, a commonly used chelator in positron emission tomography medical imaging with ⁶⁴Cu, has been synthesized using non-conventional synthetic methodologies (grinding and microwave heating) with intermediates characterized by 2D-NMR and single crystal XRD. The overall synthesis time of CB-TE2A from cyclam could be shortened to 5 days from the 35 days required for the conventional synthesis.

1. Introduction

Mechanochemistry is the application of mechanical actions, such as grinding, milling, rubbing or shearing, in order to induce a chemical transformation.¹⁻³ It is based on the principle that, once an intimate mixture of chemicals is subjected to mechanical stress, frictional energy is sufficient to overcome energy barriers and induce chemical transformations. Mechanochemical synthesis can be carried out either manually, using a mortar and a pestle, or by employing specialized grinding/milling machines.⁴ The former is the cheapest and the simplest method to set up but lacks the required reproducibility, making the outcome of the reaction dependent not only on the energetics of the chemical process, but also the physical characteristics of the manipulating chemist.^{1,2} The use of automated ball milling apparatus overcomes this problem, giving a possibility to standardize reaction conditions by controlling parameters, such as reaction time and energy transferred.^{4,5} Two different types of ball mills are commonly available in organic synthesis laboratory: vibration or mixer ball mills (MBMs) and planetary ball mills (PBMs)..A planetary ball mill was used in this work.^{6,7}



Scheme 1 - General schematic for mono- and bis-N-alkylation of tetraazamacrocycles.

In recent years, the capacity of mechanochemistry to generate complex molecules has attracted the attention of a broad audience from different research areas across organic and inorganic chemistry, including catalyst design and the preparation of pharmaceutical materials.^{8,9} The main advantages of mechanochemistry over more conventional laboratory techniques, such as the heating of solutions, are reaction

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time, reduced solvent use and efficiency of activation energy input.¹⁰⁻¹² Furthermore, simple milling protocols have been improved further by developing new methods, such as liquid or/and ion assisted grinding (LAG or ILAG),^{10,13} or the more recently developed polymer-assisted grinding (POLAG).¹⁴ Mechanochemistry has been applied for solvent-free peptide synthesis,¹⁵ preparation of mono- and bis-(thio)ureas,⁵ and optimization of multiple C-C bond forming reactions, such as Suzuki–Miyaura, Heck, and Sonogashira couplings, aldol reactions, cycloadditions, and heterocycle synthesis.¹⁶⁻¹⁸ Here we present a novel application of mechanochemistry in N-alkylation of glyoxal bridged bisaminal derivatives of cyclam (1,4,8,11-tetraazacyclotetradecane) and cyclen (1,4,7,10-tetraazacyclododecane) – macrocyclic polyamines with high affinity for various metal ions.

The affinity of tetraaza macrocycles for a given metal depends on ring size, macrocycle backbone flexibility, and the nature and number of coordinating atoms.¹⁹ Thus, different macrocyclic polyamine derivatives have been produced, exhibiting increased affinity and concurrent complex stability towards certain metal cations (transition metals or lanthanides).²⁰ Tetraazamacrocyclic complexes have found use in a range of applications, dependent on the chelated metal ion. The majority of applications relate to medical imaging agents, such as MRI contrast agents containing gadolinium(III),^{21,22} optical agents containing europium(III) and terbium(III) or near-IR emitting lanthanides,²³⁻²⁵ along with nuclear medicine probes incorporating gallium-68,^{26,27} technetium-99m^{28,29} and copper-64^{30,31}. Tetraazamacrocyclic complexes with various metals have also been used as protein binding agents,^{32,33} anti-malarial drugs^{34,35} and as catalysts,^{9,36}

Cyclam (1,4,8,11-tetraazacyclotetradecane) is one of the most used macrocyclic polyamines because it can form stable complexes with 1st row transition metal cations. Usually, metal selectivity is achieved by variation of the nature of pendant arms attached to the ring nitrogen atoms. The majority of cyclam chelator focus has been with acetate functionalized derivatives such as TETA, TE2A, and CB-TE2A.^{31,32} There is interest in the controlled synthesis of mono- or bis- substituted derivatives which may have optimized chelation properties for particular metal ions.³³ Selective step-wise functionalization to form unsymmetrical chelators is relevant to advanced chelator design.^{37,38} The increased reactivity of bisaminal cyclen leads to challenges in the synthesis of unsymmetrical chelators, the development of a method selectively functionalize would allow access to a range of novel chelators beyond the ubiquitous four arm derivative DOTA.^{39,35}

Increase of backbone rigidity of cyclam and cyclen can be achieved by including an internal bridge between adjacent (side-bridge) or non-adjacent (cross-bridge) nitrogen atoms and plays an important role in developing configurationally restricted chelators which can form metal complexes of increased stability.³⁶ The key intermediate in both cases is a glyoxal bridged bisaminal azamacrocycle which is alkylated at one or two of the aminal nitrogen atoms to include side arms carrying a functionality to improve coordination properties, such as stability, or for conjugation to a biomolecule or solid phase support.

2. Results and discussion

2.1. Alkylation of glyoxal cyclam and cyclen

Conventional solution chemistry can provide access to mono N-alkylated glyoxal-bridged macrocycles, however, this transformation requires a large excess of alkyl bromide and, dependent on reactivity of alkyl bromide, may take several days.^{40,41} The reaction is usually stopped at the mono-alkylation stage by selection of an appropriate type and amount of solvent to create reaction conditions where the precipitation of a mono-substituted product occurs.⁴² Bis N-alkylation, under conventional conditions, is more challenging due to the reduced reactivity of the second exo-nitrogen and may require up to three weeks to reach completion.^{42,43}

An initial test reaction was carried out by grinding glyoxal bridged bisaminal cyclam with a stoichiometric amount of 4-cyanobenzyl bromide in the presence of a small amount of acetonitrile (90 μ L) for a relatively short period of time (30 min.) to give a mono N-substituted macrocycle **1** in a quantitative yield (see Table 1). This discovery encouraged us to investigate this method of alkylation of cyclam using various alkyl bromides, see Scheme 1, and to extend this methodology to alkylation of 12 membered ring macrocycle analogue, cyclen.

	D 1 (Alkylation	
Br-K	Product	yield ^a , %	
	1	97 ± 2^{b}	
Br NO ₂	2	85	
Br	3	89 ± 1^{b}	
Br	4	81	
Br Br Br	5	64	
Br	6	32	

Table 1. Alkylation of glyoxal cyclam by grinding for 30 min in presence of a stoichiometric amount of alkyl bromide

^{*a*} Alkylation yield is based on a macrocycle and is calculated by dividing the mass of product(s) obtained by the theoretical mass for a given mixture of mono-armed and doubled-armed products. ^{*b*} An average of three independent experiments.

Various bromides - 4-tolyl, benzyl, 4-cyanobenzyl, 4-nitrobenzyl, 3,5-dibromobenzyl bromides and *t*butyl bromoacetate – were trialled. The choice of the alkyl bromide was guided by the potential to investigate the impact of the electronic properties of the substituents and also consideration of target molecules for future application. Of particular interest are the following: (*i*) 4-cyano and 4-nitrobenzyl substituents which can be reduced to benzylamine or aniline derivatives and the primary amine group used for a bioconjugation reaction to a protein or peptide, as a bifunctional chelator.^{23,32} (*ii*) Bis N-substituted non-functionalized benzyl and tolyl derivatives are key intermediates in cross-bridge cyclen / cyclam synthesis.^{35,44} (iii) N-tert-butyl acetate derivatives can be easily deprotected to reveal carboxylates which can either act as supplementary metal chelating groups or be used for bioconjugation reactions.^{43,45} The reactivity of benzyl bromides is governed by the nature of phenyl ring substituents which influence the electron density on benzyl carbon. Highly reactive aryl bromides carrying electron donating groups would be preferred for synthesis of bis-alkylated products, whereas less reactive electron poor bromides might be applied in the exclusive preparation of mono substituted derivatives.

Dr D	D- D	Due de st	Alkylation	
	BI-K	Product	yield ^a , %	
	Br	1	98 ± 1^{b}	
	Br NO ₂	2	98	
	Br	15	95 ± 1^{b}	
	Br	4	99 ^c	
	Br Br	5	92	
	Br	6	50	

Table 2. Alkylation of glyoxal cyclam by grinding for 3 hours in presence of a 4-fold excess of alkyl bromide

^a</sup> Alkylation yield is based on a macrocycle and is calculated by dividing the mass of product(s) obtained by the theoretical mass for a</sup>given mixture of mono-armed and doubled-armed products.^b An average of three independent experiments.^c In 2:1 mixture with 16.

Glyoxal-bridged bisaminal cyclam and cyclen were synthesized following standard literature methodology.⁴⁶ Mechanochemical alkylation of glyoxal-bridged cyclam and cyclen has been carried out using a

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planetary ball mill (PBM) apparatus under LAG conditions (90 μ L acetonitrile) at a 0.1 g scale of azamacrocycle. One arm substitution was targeted by using a stoichiometric amount of alkyl bromide and 30 mins grinding time, whilst the two arm bis-substitution reaction was attempted by employing a four-fold excess of alkyl bromide and a 3 h grinding time, see Tables 1 and 2.

		Macrocycle /		Alleylation
Br-R	Product	alkyl bromide ra-	Time	
		tio		yield, %
	1	1:2.5	2 days	78
Br NO ₂	2	1:4	24 hours	87
Br	3	1:1	16 hours	85
Br	15	1:3	24 hours	93
Br	4	1:4	16 hours	88
Br	16	1 : 16	16 days	85
Br Br	5	1:1	16 hours	82
Broo	6	1:4	16 hours	93
Broo	18	1 : 15	21 days	70

Table 3. Alkylation of glyoxal cyclam via conventional solution chemistry

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These conditions were selected after multiple trials in which reaction time, ratio of reagents and number of grinding balls were varied (data not shown). In order to confirm the reproducibility and extent of applicability of mechanochemical N-alkylation of azamacrocycles, reactions with 4-cyanobenzyl and 4tolyl bromide were repeated in triplicate. In order to separate alkylated species from unreacted azamacrocycle and/or excess of alkyl bromide, at the end of reaction, paste-like mixtures were washed multiple times with diethyl ether and tetrahydrofuran. The isolated powders were dried under vacuum, weighed and analyzed by ¹H NMR to determine their composition. In case of the mixtures obtained from tolyl substitution reactions, analytically pure samples of mono- (**3**) and bis N-substituted products (**15**) could be obtained by rewashing the dried mixtures with a minimum amount of chloroform in which monosubstituted products were highly soluble whereas bis-substituted products were almost insoluble.

Grinding of the bis-aminal cyclam with a stoichiometric amount of 4-cyanobenzyl, 4-nitrobenzyl, 4-tolyl, benzyl, 3,5-dibromobenzyl bromide or *t*-butyl bromoacetate for 30 mins produced only mono-N-substituted derivatives **1-6** with isolated yields of 98%, 85%, 90%, 81%, 64% and 34% respectively (see Scheme 1, n = 1, Table 1). The reaction of 4-tolyl benzyl bromide to form **5** was carried out with a 1:1 molar ratio for 3 hours showing no significant (< 5%) increase in yield.

An attempt was made to probe the conditions required to either (a) increase the yields of the monosubstituted products or (b) drive the reactions to form the bis-substituted compounds. Increasing the amount of alkyl bromide to 4 equivalents and the grinding time up to 3 h did not improve the near quantitative 98% yield for mono-4-cyanobenzyl substitution (1), but increased the yield of *t*-butyl bromoacetate mono-substituted product (6) to 50% and drove the 4-nitrobenzyl and 3,5-dibromobenzyl bromide mono-substitution reactions almost to completion (98% (2) and 92% (5) respectively, see Table 2). These alkyl halides did not react further to form bis-alkylated macrocycles upon increase of reaction time and/or molar ratio. The use of benzyl bromide under these conditions led to a 2:1 mixture of mono- (4) and bis- (16) N-substituted compounds, but when 4-tolyl bromide was used, exclusively bis-Nsubstituted derivative (15) was obtained in an almost quantitative 95% yield (see Table 2). It should be

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noted that, broadly, reactivity of different benzyl/alkyl bromides in mechanochemical syntheses mirrored that observed for conventional in solution reactions (see Table 3), *i.e.* even when used in excess, electron poor 4-cyano, 4-nitro and 3,5-dibromobenzyl bromides gave exclusively mono-substituted products (see Table 1 and 2) when the use of excess of 4-tolyl bromide yielded a pure bis-N-substituted product (see Table 2).

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$\begin{array}{c} 33\\ 35\\ 36\\ 37\\ 39\\ 41\\ 43\\ 44\\ 44\\ 44\\ 46\\ 55\\ 55\\ 55\\ 55\\ 55\\ 55\\ 55\\ 5$	

Table 4. Alkylation of glyoxal cyclen by grinding for 30 min in presence of a stoichiometric amount of alkyl bromide

Dr D	Draduat	Alkylation
ВГ-К	Product	yield ^a , %
Br	7	87 ± 2^{b}
Br NO ₂	8 ^c	65
Br	9 ^d	75 ± 3^{b}
Br	10 ^e	79
Br Br Br	11	60
Br	12 ^f	68

^{*a*} Alkylation yield is based on a macrocycle and is calculated by dividing the mass of product(s) obtained by the theoretical mass for a given mixture of mono-armed and doubled-armed products. ^{*b*} An average of three independent experiments. ^{*c*} In 10:1 mixture with 20. ^{*d*} In 5:1 mixture with 21. ^{*e*} In 17:1 mixture with 22. ^{*f*} In 2:1 mixture with 24.

The same methodology was subsequently applied to the synthesis of cyclen derivatives, however, a clean production of either mono- or bis-N-substituted products was rarely obtained, confirming the increased reactivity of glyoxal-bridged bisaminal cyclen to alkylation (see Scheme 1, n = 0 and Table 4 and 5). Clean mono N-substituted products were only obtained by using a short grinding time (30 min.) and stoichiometric amounts of deactivated 4-cyanobenzyl and 3,5-dibromo benzyl bromides (87% (7) and 60% (11) respectively (see Table 4). Pure bis-N-substituted products were only isolated for reactive

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4-tolyl and *t*-butyl acetyl bromides using a 4-fold excess of alkyl bromide and 3 h grinding (94% (**21**) and 86% (**24**) respectively, see Table 5). For these reasons, apart from the benefits of shortening reaction times, advantages of mechanochemical syntheses over the conventional approach are less marked. It should be noted, however, that conventional in solution syntheses suffer from some of the same limitations and pure compounds could be obtained only using electron poor 4-nitro and 3,5-dibromo benzyl bromides (**7** and **11** respectively, see Table 6).

Br-R	Product	Alkylation yield ^a , %
	19 ^c	86 ± 1^{b}
Br NO ₂	20 ^d	96
Br	21	94 ± 1^{b}
Br	22 ^e	98
Br Br	23 ^f	92
Br O	24	86

Table 5. Alkylation of glyoxal cyclen by grinding for 3 hours in presence of a 4-fold excess of alkyl bromide

^{*a*} Alkylation yield is based on a macrocycle and is calculated by dividing the mass of product(s) obtained by the theoretical mass for a given mixture of mono-armed and doubled-armed products. ^{*b*} An average of three independent experiments. ^{*c*} In 1:8 mixture with 7. ^{*d*} In 3:1 mixture with 8, ^{*e*} In 1:1 mixture with 10. ^{*f*} In 4:1 mixture with 11.

A comparison between the reactivity of bisaminal cyclam and cyclen towards N-alkylation under LAG conditions reveals that, as expected, the cyclen derivative is more reactive than the cyclam derivative, due to the rigidified nature of the macrocycle influencing the orientation of the exo nitrogen atoms. As mentioned, for cyclen derivatives, pure mono-substituted products were only available from deactivated 4-cyanobenzyl and 3,5-dibromobenzyl bromides reactions (7 and 11 respectively). However, this increased reactivity allowed preparation of pure bis-substituted bisaminal cyclen using electronically neutral *t*-butyl bromoacetate (24), which was not possible in the case of the cyclam equivalent.

	Droduat	Alkylation
DI-K	Floduct	yield ^b , %
Br	7 ^d	72
Br NO ₂	8	88± 1 ^c
Br	9 ^e	77
Br	$10^{\rm f}$	72
Br Br	11	84 ± 1^{c}
Br	12 ^g	60

Table 6. Alkylation of glyoxal cyclen via conventional solution chemistry.^a

^{*a*} All reactions carried out for 24 hours. ^{*b*} Alkylation yield is based on a macrocycle and is calculated by dividing the mass of product(s) obtained by the theoretical mass for a given mixture of mono-armed and doubled-armed products. ^{*c*} An average of two independent experiments. ^{*d*} In 5:1 mixture with **19**. ^{*e*} In 6:1 mixture with **21**. ^{*f*} In 8:1 mixture with **22**. ^{*g*} In 4:1 mixture with **24**.

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2.2. Efficient synthesis of CB-cyclam

Due to the increased kinetic stability of their metal complexes, cross-bridged cyclam derivatives are preferred candidates for radiometal chelation in biomedical applications, especially in the use of copper(II) radioisotopes such as copper-64.⁴⁷ Two general methods are used to symmetrically Nfunctionalize the cross-bridged cyclam azamacrocycle. The first procedure involves the addition of the selected pendent arms onto the tetracyclic bisaminal directly at the start of the synthesis followed by reductive ring cleavage to give the desired cross-bridged species. This strategy, however, is limited to the specific cases when a sufficiently reactive side arm precursor is available due to the lower reactivity of the second aminal nitrogen atom in cyclam.^{40,48} The second strategy relies on the initial preparation of an unsubstituted cross-bridged cyclam (by using a highly reactive side arm which is removed after the cross-bridge formation by catalytic hydrogenation) which is then functionalized with the desired pendent arm(s). Thus, the second approach is much more versatile and is generally preferred.⁴⁰

CB-TE2A (Scheme 2) is a widely used copper(II) chelator consisting of a cross-bridged cyclam N-substituted by two methylcarboxylic acid pendent arms.^{40,49} It has been specifically developed to form stable *in vivo* complexes with copper(II) for ⁶⁴Cu PET imaging and offers significant stability advantages over unbridged analogues.^{50,51} CB-TE2A is generally prepared following the method originally proposed by Weisman and co-workers.⁴⁰ First, unsubstituted cross-bridged cyclam is prepared by reduction and hydrolysis of a bis-benzyl aminal intermediate which is then reacted with ethyl bromoacetate yielding the bis-substituted cross-bridged species. Finally, the ethyl ester protecting groups are subjected to acid hydrolysis, revealing the carboxymethyl arms of CB-TE2A which improve the metal ion coordination by offering additional donor atoms to give highly stable complexes.



The synthesis of CB-TE2A by conventional methods is a time consuming six step process taking 35 days with an average 45% yield (calculated from cyclam). The most time consuming steps are glyoxalbridged cyclam alkylation (16 days for benzyl bromide) and reductive ring opening in presence of a large excess of NaBH4 (50 eq., 14 days).⁴⁰ We believed that by using the alkylation method described in this work we could dramatically reduce the overall time spent in preparation of CB-cyclam, a key intermediate in CB-TE2A synthesis. As the bis-N-benzyl substitution is only required to direct the opening of the bridged system towards CB-cyclam, in our synthesis we replaced benzyl bromide by more reactive tolyl bromide (see Scheme 2). Mechanochemical alkylation requires only 4-fold excess of tolyl bromide and is complete in 3 hours (**15**, Scheme 1, Table 2) giving a 95% yield which is a significant increase in efficiency when compared with the 16 day reaction using benzyl bromide following conventional methodology.

As 15 is a novel key intermediate, further analysis was carried out in order to fully characterise its structure. Full assignment of the NMR was carried out using 2D-NMR (¹H-DQF-COSY) and compared with spectra of analogous reported compounds and the mono-substituted derivative (**3**) (full details are reported in the ESI). In order to determine the structure of **15** in the solid state, single crystals of **15** and **21**, suitable for single-crystal X-ray structure determination, were obtained within 2-7 days from vapour diffusion of diethyl ether into a methanolic solution at room temperature. The X-ray crystal structures confirm the identity of **15** and **21** (see ESI) and show that the cis geometry has been adopted around the bisaminal bridge, as expected from the synthetic method used. The two 'exo' nitrogen atoms, where the lone pairs points out of the central cavity, have been alkylated to form quaternized derivatives. The full details of the structural data of **15** and the two crystalline forms of compound **21** are presented in the

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supplementary data. A useful comparison of the relative strains on the alkylated bisaminal macrocycles can be obtained by examining the bond angles around the benzyl substituted nitrogens. The 14membered cyclam ring (**15**) has bond angles in the range 107.5-112.6° around the quaternized ring nitrogen and the equivalent positions in the 12-membered cyclen derivative (form II of **21**) show more flexibility with a wider range of angles from 101.5- 113.3°. This matches with the reactivity observed in the formation of these products

After reducing alkylation time from 16 days to 3 hours we looked for ways to increase the efficiency of reductive ring cleavage leading to formation of the cross-bridged derivative. We found that using only ten molar equivalents of sodium borohydride and heating the reaction by microwave irradiation we could reduce the reaction time from 14 days to 30 minutes and form **25** in an 83% yield. Given that the intermediate **25** carries tolyl rather than the traditional benzyl arms, we also confirmed effectiveness of catalytic hydrogenation and obtained expected CB-cyclam (**26**) in an 80% yield. Therefore, the demonstrated optimization of the first two steps employing non-conventional synthesis methods could shorten preparation of CB-TE2A to less than one week, which would represent a five-fold acceleration in comparison to the conventional five week synthesis time (see Scheme 2).

3. Conclusion

The rapid synthesis of different glyoxal-bridged cyclen and cyclam derivatives has been achieved using mechanochemical activation under liquid assisted grinding (LAG) conditions. To the best of our knowledge, this is the first report of preparation of mono- and/or bis-N-substituted glyoxal-bridged tetraazamacrocyclic derivatives which, after a further transformation, could give access to a variety of interesting bifunctional chelators. Using a stoichiometric ratio of reagents, mono-N-alkylated products were obtained with short reaction times and in moderate to high yields. The amount of bis-N-alkylated products in the final reaction mixtures can be increased by selecting electronically activated alkyl bromides, increasing the reagent ratio and prolonging the grinding time. The syntheses were easy to execute and often approached quantitative yields. Furthermore, the ability to stop the reaction at the mono alkylation stage is very important and permits application of this technique to the design of more sophisticated molecules. In the field of functionalized azamacrocycle preparation, these findings present grinding methodology as an attractive alternative to more conventional solution-based synthetic methods. As a proof of principle, mechanochemical activation together with a rapid microwave synthetic step has been used for preparation of cross-bridged cyclam, a key intermediate in the synthesis of CB-TE2A. The novel synthetic route showed a significant increase in efficiency resulting in a five-fold reduction in the synthesis time.

4. Experimental

4.1. Materials and methods

The starting glyoxal-bridged bisaminal cyclam and cyclen were prepared following published protocols.⁵² Solvents used were of reagent grade. Grinding experiments were carried out in a Retsch PM100 (Retsch (U.K.) Limited) planetary ball mill using a 12 mL stainless steel grinding jar and a single 10 mm diameter stainless steel ball at 400 RPM. Dry acetonitrile was used as a liquid phase throughout all Liquid Assisted Grinding (LAG) experiments. All reactions were run at room temperature (r.t.) unless otherwise stated. Microwave assisted reduction was carried out in 35 mL reactor using CEM Discovery (CEM Microwave Technology Ltd) apparatus with temperature measured via external infrared sensor. Bulk solvent was removed by evaporation under reduced pressure and trace solvent was removed using a Schlenk line. ¹H-NMR and ¹³C-NMR were obtained at 400 MHz for ¹H spectra and 100 MHz for ¹³C spectra, and were referenced against standard internal TMS or residual non deuterated solvent signal. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), m (multiplet) and dt (double triplet). When a mixture of products is present, a ratio of characteristic aminal protons in ¹H-NMR is used to determine ratios and calculate yields. Double-Quantum Filtered Correlation Spectroscopy (¹H-DOF-COSY) was carried out on 3 and 15. High and low resolution mass spectra were recorded using an electrospray ion-trap LC-MS in positive mode. Single crystal X-ray diffraction data were collected using an imaging plate diffractometer operating with Mo radiation. Routine data collection and processing pro-

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cedures were adopted. CCDC 1417374-1417376 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033).

4.2. Synthesis

General procedure for mono- and bis-N-alkylation of glyoxal-bridged tetraazamacrocycles via grinding

A mixture of alkylbromide and glyoxal-bridged cyclam or cyclen in 1:1 molar ratio was ground in the presence of dry acetonitrile (90 μ l) for 30 min (for one arm) and in 1:4 molar ratio for 3 h (for two arms). The crude products were scraped off the walls of the grinding jar and were washed multiple times with diethyl ether and tetrahydrofuran (THF) to remove un-reacted starting materials and give expected mono- or bis-N-substituted products (pure or in mixtures) in powdery form.

General procedure for mono- and bis-N-alkylation of glyoxal-bridged cyclam or cyclen via a conventional in solution method

A selected alkylbromide and glyoxal-bridged cyclam or cyclen in 1:4 molar ratio was stirring for the tabulated time in dry acetonitrile (25 mL). The precipitated products were isolated by filtration and washed multiple times with diethyl ether and tetrahydrofuran (THF) to remove un-reacted starting materials.

13.1 Hz, 1H), 4.29 (d, J = 1.6 Hz, 1H), 4.75 (d, J = 13.1 Hz, 1H), 5.18 (d, J = 13.1 Hz, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.87 (d, J = 8.4 Hz, 2H).

10a-(4-nitrobenzyl)dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium bromide (**2**), first reported by Plutnar *et al.*⁵³: From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and 4- (bromomethyl)nitrobenzene (97 mg, 0.45 mmol, 1 eq.). White solid (168 mg, 85%). From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and 4- (bromomethyl)nitrobenzene (389 mg, 1.8 mmol, 4 eq.). White solid (212 mg, 98%). ¹H NMR (400 MHz, D₂O, δ): 1.45 (d, J = 14.9 Hz, 1H), 1.77 (d, J = 14.9 Hz, 1H), 2.08-2.36 (m, 3H), 2.43-2.52 (m, 2H), 2.63 (td, J = 3.5 Hz, J = 13.0 Hz, 1H), 2.95-3.18 (m, 7H), 3.19-3.38 (m, 2H), 3.44-3.64 (m, 2H), 3.71 (d, J = 2.0 Hz, 1H), 4.15-4.27 (m, 1H), 4.34 (d, J = 2.0 Hz, 1H), 4.83 (d, J = 13.3 Hz, 1H), 5.26 (d, J = 13.3 Hz, 1H), 7.76 (d, J = 8.8 Hz, 2H).

10a-(4-methylbenzyl)dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium bromide (**3**): From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and 1-(bromomethyl) 4methylbenzene (83 mg, 0.45 mmol, 1 eq.). The crude solid was purified by washing with chloroform to yield a soluble product which was dried *in vacuo* to yield a white solid (166 mg, 90%). ¹H NMR (400 MHz, D₂O, δ): 1.43 (d, J = 14.1 Hz, N-β-CH₂, 1H), 1.75 (d, J = 14.1 Hz, N-β-CH₂, 1H), 2.02-2.29 (m, N- α -CH₂, 3H), 2.36 (s, Ar CH₃, 3H), 2.38-2.48 (m, N- β -CH₂, 2H), 2.60 (td, J = 3.7 Hz, J = 12.7 Hz, N- α -CH₂, 1H), 2.92-3.13 (m, N- α -CH₂, 7H), 3.14-3.36 (m, N- α -CH₂, 2H), 3.40-3.56 (m, N- α -CH₂, 2H), 3.62 (d, J = 1.8 Hz, CH, 1H), 4.14 (td, J = 4.1 Hz, J = 13.4 Hz, N- α -CH₂, 1H), 4.33 (d, J = 1.8 Hz, CH, 1H), 4.73 (d, J = 13.5 Hz, Ar CH₂, 1H), 4.96 (d, J = 13.5 Hz, Ar CH₂, 1H), 7.35 (d, J = 8.4 Hz, Ar H, 2H), 7.39 (d, J = 8.4 Hz, Ar H, 2H). ¹³C {¹H}NMR (100 MHz, D₂O, δ): 18.0 (CH₂), 18.4 (CH₂), 20.4 (CH₃), 42.0 (CH₂), 46.6 (CH₂), 48.6 (CH₂), 51.3 (CH₂), 52.0 (CH₂), 53.3 (CH₂), 54.0 (CH₂), 59.7 (CH₂), 62.5 (CH₂), 69.6 (CH), 81.7 (CH), 122.5 (C), 129.9 (CH), 133.2 (CH), 141.9 (CH). HRMS: calc. for C₂₀H₃₁N₄: 327.2543, found: 327.2548. Elemental analysis calc. for C₂₀H₃₁BrN₄.2H₂O: C, 54.17; H, 7.96; N, 12.64%; found: C, 54.16; H, 8.22; N, 12.82. m.p. 144-145°C.

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10a-benzyldodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium bromide (**4**), first reported by Silversides *et al.*³⁷: From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and benzyl bromide (77 mg, 0.45 mmol, 1 eq.). White solid (143 mg, 81%). From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and benzyl bromide (308 mg, 1.8 mmol, 4 eq.). White solid, obtained in 2:1 mixture with **16** (175 mg, 99%). ¹H NMR (400 MHz, D₂O, δ): 1.39 (d, J = 14.5 Hz, 1H), 1.70 (d, J = 14.5 Hz, 1H) 2.02-2.26 (m, 3H), 2.33-2.48 (m, 2H), 2.56 (td, J = 4.0 Hz, J = 13.5 Hz, 1H), 2.86-3.11 (m, 7H), 3.13-3.33 (m, 2H), 3.37-3.56 (m, 2H), 3.59 (d, J = 1.5 Hz, 1H), 4.06-4.18 (m, 1H), 4.30 (d, J = 1.5 Hz, 1H), 4.72 (d, J = 13.5 Hz, 1H), 4.99 (d, J = 13.5 Hz, 1H), 7.43-7.58 (m, 5H).

10a-(3,5-dibromobenzyl)dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium bromide (**5**): From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and 1,3-dibromo-5-(bromomethyl)benzene (127 mg, 0.45 mmol, 1 eq.). White solid (159 mg, 64%). From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and 1,3-dibromo-5-(bromomethyl)benzene (508 mg, 1.8 mmol, 4 eq.). White solid (229 mg, 92%). ¹H NMR (400 MHz, D₂O, δ): 1.39 (d, *J* = 14.4 Hz, 1H), 1.73 (d, *J* = 14.4 Hz, 1H) 2.02-2.32 (m, 3H), 2.36-2.49 (m, 2H), 2.55 (td, *J* = 3.0 Hz, *J* = *I*3.0 Hz, 1H), 2.85-3.21 (m, 8H), 3.31 (d, *J* = 11.5 Hz, 1H), 3.36-3.55 (m, 2H), 3.63 (d, *J* = 1.1 Hz, 1H), 4.08-4.22 (m, 1H), 4.24 (d, *J* = 1.1 Hz, 1H), 4.64 (d, *J* = 13.4 Hz, 1H), 5.03 (d, *J* = 13.4 Hz, 1H), 7.64 (d, *J* = 1.0 Hz, 2H), 7.91-7.99 (m, 1H). ¹³C{¹H}NMR (100 MHz, D₂O, δ): 18.0, 18.4, 41.9, 46.5, 48.6, 51.3, 51.9, 53.3, 53.9, 60.2, 69.6, 82.5, 123.2, 129.3, 134.7, 136.6. HRMS: calc. for C₁₉H₂₇N₄Br2: 471.0581, found 471.0577. Elemental analysis calc. for C₁₉H₂₇Br₃N₄: C, 41.40; H, 4.94; N, 10.17%; found: C, 41.79; H, 5.12; N, 10.01. m.p. 178-179°C.

10a-(2-(tert-butoxy)-2-oxoethyl)dodecahydro-1H-3a,5a,8a,10a-tetraazapyren-10a-ium bromide (6), first reported by Silversides *et al.*⁵⁴: From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and t-butyl bromoacetate (88 mg, 0.45 mmol, 1 eq.). White solid (60 mg, 32%). From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.515 mmol, 1 eq.) and t-butyl bromoacetate (351 mg,

1.8 mmol, 4 eq.). White solid (94 mg, 50%). ¹H NMR (400 MHz, D₂O, δ): 1.42 (d, J = 13.0 Hz, 1H), 1.56 (s, 9H) 1.90 (d, J = 13.0 Hz, 1H), 2.12-2.29 (m, 1H), 2.30-2.62 (m, 5H), 2.83-3.21 (m, 7H), 3.49 (t, J = 11.2 Hz, 1H), 3.64 (d, J = 12.7 Hz, 1H), 3.81 (t, J = 11.2 Hz, 1H), 3.91-4.10 (m, 3H), 4.30-4.49 (m, 2H), 4.67 (d, J = 15.6 Hz, 1H).

8a-(4-cyanobenzyl)decahydro-1H-2a, *4a*, *6a*, *8a-tetraazacyclopenta[fg]acenaphthylen-8a-ium bromide* (7): From cis-13-1,4,7,10- tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and 4-cyanobenzylbromide (101 mg, 0.515 mmol, 1 eq.). White solid (178 mg, 89%). From cis-13-1,4,7,10- tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and 4-cyanobenzylbromide (404 mg, 2.06 mmol, 4 eq.) White solid (135 mg, 72%). ¹H NMR (400 MHz, D₂O, δ): 2.45-2.62 (m, 2H), 2.73-2.98 (br m, 4H), 3.05-3.35 (br m, 5H), 3.43-3.65 (m, 4H), 3.77 (d, *J* = 1.8 Hz, 1H), 4.06 (d, *J* = 1.8 Hz, 1H), 4.13-4.29 (m, 1H), 4.74 (d, *J* = 13.5 Hz, 1H), 4.99 (d, *J* = 13.5 Hz, 1H), 7.76 (d, *J* = 8.2 Hz, 2H), 7.91 (d, *J* = 8.2 Hz, 2H) ¹³C{¹H}-NMR (100 MHz, D₂O, δ): 43.7, 47.5, 47.6, 48.2, 48.2, 51.3, 57.1, 60.6, 61.5, 71.6, 83.1, 113.8, 118.6, 132.0, 133.1, 133.5. HRMS: calc. for C₁₈H₂₄N₅⁺: 310.2026; found: 310.2022. Elemental analysis calc. for C₁₈H₂₄BrN₅: C, 55.39; H, 6.20; N, 17.94%; found: C, 55.18; H, 6.04; N, 17.73, m.p. 200–202 °C.

8a-(4-nitrobenzyl)decahydro-1H-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylen-8a-ium bromide $al.^{42}$: (8), Rohovec From first reported by et cis-13-1.4.7.10tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and 4-nitrobenzylbromide (111 mg, 0.515 mmol, 1 eq.). White solid, obtained in 10:1 mixture with **20** (138 mg, 65%). From cis-13-1,4,7,10-tetraazatetracyclo $[5.5.1.0^{4,14}0^{10,13}]$ tetradecane (100 mg, 0.515 mmol, 1 eq.) and 4nitrobenzylbromide (445 mg, 2.06 mmol, 4 eq.). White solid (175 mg, 89%). ¹H NMR (400 MHz, D₂O, δ): 2.33-2.46 (m, 2H), 2.59-2.73 (br m, 3H), 2.74-2.84 (m, 1H), 2.94-4.03 (m, 1H), 3.10-3.22 (m, 3H), 3.28-3.39 (m, 1H), 3.40-3.59 (m, 4H), 3.65 (d, J = 2.3 Hz, 1H), 3.94 (d, J = 2.3 Hz, 2H), 4.03-4.13 (m, 1H), 4.88 (d, J = 13.5 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 8.2 (d, J = 8.5 Hz, 2H).

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8a-(4-methylbenzyl)decahydro-1H-2a, 4a, 6a, 8a-tetraazacyclopenta[fg] acenaphthylen-8a-ium bromide(9): From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.)and 4-methylbenzylbromide (95 mg, 0.515 mmol, 1 eq.). White solid, obtained in 5:1 mixture with**21** (153 mg, 78%). From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg,0.515 mmol, 1 eq.) and 4-methylbenzylbromide (380 mg, 2.06 mmol, 4 eq.). White solid, obtained in6:1 mixture with**21**(152 mg, 77%).

a-benzyldecahydro-1H-2a, 4*a*, 6*a*, 8*a-tetraazacyclopenta[fg]acenaphthylen-8a-ium bromide* (10): From cis-13-1,4,7,10-tetraazatetracyclo[$5.5.1.0^{4,14}0^{10,13}$]tetradecane (100 mg, 0.515 mmol, 1 eq.) and benzylbromide (88 mg, 0.515 mmol, 1 eq.). White solid, obtained in 17:1 mixture with **22** (149 mg, 79%). From cis-13-1,4,7,10- tetraazatetracyclo[$5.5.1.0^{4,14}0^{10,13}$]tetradecane (100 mg, 0.515 mmol, 1 eq.) and benzylbromide (352 mg, 2.06 mmol, 4 eq.). White solid, obtained in 8:1 mixture with **22** (128 mg, 72%).

8a-(3,5-dibromobenzyl)decahydro-1H-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylen-8a-ium

bromide (11): From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and 1,3-dibromo-5-(bromomethyl)benzene (145 mg, 0.515 mmol, 1 eq.). White solid (162 mg, 60%). From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and 1,3-dibromo-5-(bromomethyl)benzene (580 mg, 2.06 mmol, 4 eq.). White solid (210 mg, 85%). ¹H NMR (400 MHz, D₂O, δ): 2.42-2.56 (br m, 2H), 2.70-2.86 (m, 3H), 2.84-2.98 (br m, 1H), 3.00-3.18 (m, 2H), 3.20-3.34 (m, 3H), 3.40-3.51 (m, 1H), 3.51-3.64 (m, 3H), 3.71 (d, *J* = 2.5 Hz, 1H), 4.00 (d, *J* = 2.5 Hz, 1H), 4.08-4.20 (m, 1H), 4.63 (d, *J* = 13.4 Hz, 1H), 4.82 (d, *J* = 13.4 Hz, 1H), 7.71 (d, *J* = 1.6 Hz, 2H), 7.96 (t, *J* = 1.6 Hz, 1H); ¹³C{¹H}-NMR (100 MHz, D₂O, δ): 43.7, 47.4, 47.6, 48.2, 48.2, 51.2, 57.1, 59.9, 61.4, 71.6, 83.0, 123.4, 130.4, 134.0, 136.6. HRMS: calc. for C₁₇H₂₃Br₂N₄⁺: 441.0284, found: 441.0277. Elemental analysis calc. for C₁₇H₂₃Br₃N₄: C, 39.03; H, 4.43; N, 10.71; found: C, 39.01; H, 4.35; N, 10.66, m.p. dec. at 187 °C.

8a-(2-(tert-butoxy)-2-oxoethyl)dodecahydro-2a, 4a, 6a, 8a-tetraazacyclopenta[fg]acenaphthylene-8aium bromide (12): From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]-tetradecane (100 mg,0.515 mmol, 1 eq.) and*t*-butyl bromoacetate (101 mg, 0.515 mmol, 1 eq.). White solid, obtained in 2:1mixture with 24 (127 mg, 68%). From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane(100 mg, 0.515 mmol, 1 eq.) and 4-methylbenzylbromide (380 mg, 2.06 mmol, 4 eq.). White solid, obtained in 4:1 mixture with 24 (113 mg, 60%).

5a, *10a-bis-(4-methylbenzyl)tetradecahydro-3a*, *5a*, *8a*, *10a-tetraazapyrene-5a*, *10a-diium bromide (15)*, first reported by Silversides *et al*.⁴³: From cis-3a, *5a*, *8a*, *10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and 4-methylbenzylbromide (333 mg, 1.8 mmol, 4 eq.)*. The crude solid was purified by washing with chloroform to yield a white insoluble solid (253 mg, 95%). ¹H NMR (400 MHz, D₂O, δ): 1.87 (d, J = 15.4 Hz, N-β-CH₂, 2H), 2.14-2.32 (m, N- α -CH₂, 2H), 2.37(s, Ar CH₃, 6H), 2.81 (td, J = 3.5 Hz, J = 12.5 Hz, N- β -CH₂, 2H,), 3.18 (d, J = 13.0 Hz, N- α -CH₂, 4H), 3.34-3.59 (m, N- α -CH₂, 2H), 3.63-3.77 (m, N- α -CH₂, 4H), 3.72 (td, J = 4.0 Hz, J = 12.6 Hz, N- α -CH₂, 2H), 4.34 (td, J = 5.5 Hz, J = 13.0 Hz, N- α -CH₂, 2H), 4.70 (d, J = 13.0 Hz, Ar CH₂, 1H), 5.08 (s, CH, 2H), 5.22 (d, J = 13.0 Hz, Ar CH₂, 2H), 7.37 (d, J = 7.8 Hz, Ar H, 4H), 7.46 (d, J = 7.8 Hz, Ar H, 4H).

3a,8a-dibenzyltetradecahydro-3a,5a,8a,10a-tetraazapyrene-3a,8a-diium bromide (*16*), first reported by Weisman *et al.*⁴⁰: From cis-3a,5a,8a,10a-tetraazaperhydropyrene (100 mg, 0.45 mmol, 1 eq.) and benzyl bromide (308 mg, 1.8 mmol, 4 eq.). White solid, obtained in a 1:2 mixture with **4**. (127 mg, 50%)

4a,8a-bis-(4-cyanobenzyl)dodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylene-4a,8a-diium bromide (**19**): From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and 4-cyanobenzylbromide (404 mg, 2.06 mmol, 4 eq.). White solid, obtained in 1:8 mix-ture with **7** (262 mg, 87%).

4a,8a-bis-(4-nitrobenzyl)dodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylene-4a,8adiium bromide (**20**): From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg,

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0.515 mmol, 1 eq.) and 4-nitrobenzylbromide (445 mg, 2.06 mmol, 4 eq.). White solid, obtained in 3:1 mixture with **8** (310 mg, 96%).

a,8*a*-*bis*-(4- methylbenzyl)dodecahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopenta[fg] acenaphthylene-4*a*,8*a*diium bromide (**21**): From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and 4-methylbenzylbromide (381 mg, 2.06 mmol, 4 eq.). White solid (253 mg, 94%). ¹H NMR (400 MHz, D₂O, δ): 2.36 (s, 6H), 3.03-3.12 (br m, 2H), 3.37 (d, *J* = 7.0 Hz, 4H), 3.50-3.66 (br m, 6H), 3.76-3.88 (m, 2H), 4.19-4.30 (m, 2H), 4.71 (d, *J* = 13.3 Hz, 2H), 4.88 (d, *J* = 13.3 Hz, 2H), 7.36 (d, *J* = 7.7 Hz, 4H), 7.45 (d, *J* = 7.7 Hz, 4H); ¹³C NMR (100 MHz, D₂O, δ): 20.4, 42.8, 46.1, 54.8, 60.8, 60.9, 77.4, 123.1, 130.3, 132.2, 142.2. HRMS: calc. for C₂₆H₃₆N₄²⁺: 202.1465, found: 202.1473. Elemental analysis calc. for C₂₆H₃₆Br₂N₄.2H₂O: C, 52.01; H, 6.71; N, 9.33; found: C, 51.98; H, 6.65; N, 9.09. m.p. 182–185 °C.

4a,8a-bis-benzyldodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg]acenaphthylene-4a,8a-diium bromide (22): From cis-13-1,4,7,10-tetraazatetracyclo[$5.5.1.0^{4,14}0^{10,13}$]tetradecane (100 mg, 0.515 mmol, 1 eq.) and benzylbromide (352 mg, 2.06 mmol, 4 eq.). White solid, obtained in 1:1 mixture with 10 (270 mg, 98%).

4a,8a-bis-(3,5-dibromobenzyl)dodecahydro-2a,4a,6a,8a-tetraazacyclopenta[fg] acenaphthylene-

4a,8a-diium bromide (**23**): From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and 1,3-dibromo-5-(bromomethyl)benzene (677 mg, 2.06 mmol, 4 eq.). White solid, obtained in 4:1 mixture with **11** (404 mg, 92%).

4a,8a-bis(2-(tert-butoxy)-2-oxoethyl)dodecahydro-2a,4a,6a,8a-

tetraazacyclopenta[fg] acenaphthylene-4a,8a-diium bromide (**24**), first reported by Oukhatar *et al.*⁵⁵: From cis-13-1,4,7,10-tetraazatetracyclo[5.5.1.0^{4,14}0^{10,13}]tetradecane (100 mg, 0.515 mmol, 1 eq.) and tbutyl bromoacetate (402 mg, 2.06 mmol, 4 eq.). White solid (260 mg, 86%). ¹H NMR (400 MHz, D₂O, δ): 1.47 (s, 18H), 2.92-3.03 (m, 2H), 3.03 -3.16 (m, 2H), 3.36 (d, J = 13.9 Hz, 2H), 3.53-3.64 (m, 2H), 4.00-4.13 (m, 6H,), 4.17 -4.27 (m, 2H), 4.41 (d, J = 16.7 Hz, 2H), 4.51 (s, 2H), 4.64 (d, J = 16.7 Hz, 2H). ¹³C NMR (100 MHz, D₂O, δ): 27.1, 42.9, 47.0, 56.9, 57.2, 64.0, 79.1, 87.4, 163.3. HRMS: calc. for C₂₂H₄₀N₄O₄ Br⁺: 503.2227, found: 503.2228. Elemental analysis calc. for C₂₂H₄₀Br₂N₄.O₄.4H₂O: C, 40.25; H, 7.37; N, 8.53; found: C, 40.77; H, 7.29; N, 8.75. m.p. 125-127 °C.

4,11-bis(4-methylbenzyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**25**), first reported by Silversides *et al.*⁴³: To a stirred solution of 5a,10a-bis-(4-methylbenzyl)tetradecahydro-3a,5a,8a,10a-tetraazapyrene-5a,10a-diium bromide (**15**) (100 mg, 0.168 mmol) in ethanol (10 mL) was added slowly sodium boro-hydride (317 mg, 8.3 mmol) and the mixture was stirred for 30 min at r.t. before heated using micro-wave irradiation at 100°C for 30 min. After cooling to r.t., water (10 mL) was added to decompose excess NaBH₄ and solvents were removed under vacuum. Water (30 mL) was added to the residue, the solution was made basic by addition of excess of solid KOH (pH 14), and extracted with dichloromethane (4 x 30 mL). Combined organic extracts were dried and solvent was removed to yield a white solid (139 mg, 83%). ¹H NMR (400 MHz, CDCl₃, δ): 1.33-1.43 (m, 2H), 1.52-1.63 (m, 2H), 2.30 (s, 6H), 2.35-2.37 (m, 8H), 2.46-2.51 (m, 3H) 2.8-2.9 (m, 2H), 3.15 (d, J = 13.5 Hz, 2H), 3.21 (d, J = 8.6 Hz, 2H), 3.74 (d, J = 13.5 Hz, 2H), 3.89-4.00 (m, 2H), 7.12 (d, J = 8.0 Hz, 4H), 7.23 (d, J = 8.0 Hz, 4H).

1,4,8,11-tetra azabicyclo [6.6.2] hexadecane (**26**), first reported by Weisman et al.⁴¹: A solution of **25** (200 mg, 0.46 mmol) in glacial acetic acid (20 mL) was added to a stirred suspension of 10% palladium on carbon (25 mg) in glacial acetic acid (2 mL) and the reactor was flushed with nitrogen and hydrogen before filling with hydrogen. The mixture was stirred at r.t. under 1 bar of hydrogen for 24 h. Then, the reactor was flushed with nitrogen and the reaction mixture was filtered through a pad of Hyflo filtration aid and washed with glacial acetic acid (3 x 15 mL). Solvents were removed under vacuum and the residue dried to yield a yellow oil (83 mg, 80%). ¹H NMR (400 MHz, C₆D₆, δ): 1.11-1.23 (m, 2H), 1.40-1.54 (m, 2H), 2.00-2.08 (m, 2H), 2.09-2.19 (m, 12H), 2.22 -2.30 (m, 2H), 2.40-2.56 (m, 6H), 2.72-2.86 (m, 4H), 2.13-2.26 (m, 2H), 3.55 (br s, 2H).

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ASSOCIATED CONTENT

Supporting Information: NMR spectra and crystallographic data. This material is available free of

charge via the internet at http://pubs.acs.org.

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