LUND UNIVERSITY

Flexible Synthesis of Rigid Cyclophanes - Synthesis and derivatisation of 2,7-diaza-1,2,3,6,7,8-hexahydropyrene into chiral macrocycles

Toftered, Jörgen

2004

Link to publication

Citation for published version (APA):
Toftered, J. (2004). *Flexible Synthesis of Rigid Cyclophanes - Synthesis and derivatisation of 2,7-diaza-1,2,3,6,7,8-hexahydropyrene into chiral macrocycles*. [Doctoral Thesis (compilation), Centre for Analysis and Synthesis]. Bioorganic Chemistry, Lund University.

Total number of authors:
1

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Flexible synthesis of rigid cyclophanes

Synthesis and derivatisation of 2,7-diaza-1,2,3,6,7,8-hexahydropyrene into chiral macrocycles

Jörgen Toftered

Bioorganic Chemistry
Lund
2004

LUND INSTITUTE OF TECHNOLOGY
Lund University

Akademisk avhandling som för avläggandet av teknologie doktorsexamen vid tekniska fakulteten vid Lunds Universitet kommer att offentligen försvaras på Kemicentrum, sal K:D, fredagen den 10 december, kl. 13.30.

A doctoral thesis at a university in Sweden is produced as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarises the accompanying papers. These have either already been published or are manuscripts at various stages (*in press, submitted* or *in manuscript*).

Jörgen Toftered
Bioorganic Chemistry
Center for Chemistry and Chemical Engineering
Lund University
P.O. Box 124
S-221 00 Lund
Sweden

ISBN 91-628-6341-X

Printed by Media-tryck, Lunds Universitet

Beer is proof that God loves us and wants us to be happy.

Benjamin Franklin (1706-1790)

Organization	Document name
LUND UNIVERSITY Bioorganic Chemistry P.O. Box 124 S-221 00 Lund Sweden	DOCTORAL DISSERTATION
	Date of issue 2004-11-16
Author(s) Jörgen Toftered	Sponsoring organization Swedish Research Council, Swedish Foundation for Strategic Research and the program "Glycoconjugates in Biological Systems" sponsored by the Swedish Foundation for Strategic Research

Title and subtitle: Flexible Synthesis of Rigid Cyclophanes - Synthesis and derivatisation of 2,7-diaza-1,2,3,6,7,8-hexahydropyrene into chiral macrocycles

Abstract

Rigid, chiral and amphiphilic cyclophanes of potential use as biomimetic host molecules were prepared by cyclic dimerisation of chiral diamino buidling blocks with amino-reactive cross-linking reagents.

A reliable synthesis of the scaffold 2,7-diaza-1,2,3,6,7,8-hexahydropyrene was developed. Chiral bidentate building blocks were formed by acylation of the aromatic diamine 2,7-diaza-1,2,3,6,7,8-hexahydropyrene with L-amino acids.

1,5-Difluoro-2,4-dinitrobenzene was employed as a cross-linking reagent, stepwise nucleophilic aromatic substitution with bidentate building blocks gave rise to a range of macrocyclic bis-dinitrodianilines.

Macrocyclic bis-ureas were formed by using substituted phenyl chloroformates as cross-linking reagents. The substitution pattern of the phenyl chloroformates was optimised for the synthesis of macrocycles. This investigation favoured phenyl chloroformates substituted with electron-withdrawing groups of intermediate power.

Applying the results from the investigation of phenyl chloroformate substitutents, macrocyclic bis-thioureas were prepared by using substituted phenyl chlorothionoformates as cross-linking reagents.

The synthesis of chiral macrocycles was further diversified by incorporating other aromatic diamines as scaffolds, namely *p*-xylylenediamine and bis-2,3:6,7-iminodimethylene-anthracene.

Future development and application of the macrocycles was discussed with emphasis on rational design based on conformational analysis.

Key words: Cyclophanes, macrocycles, cyclodimerisation, difluorodinitrobenzene, urea, thiourea, cross-linking, biomimetic

Classification system and/or index termes (if any):

Supplementary bibliographical information:	Language English
ISSN and key title:	ISBN 91-628-6341-X

Recipient´s notes	Number of pages 84	Price
	Security classification	

Distribution by (name and address) Bioorganic Chemistry, P.O. Box 124, S-221 00 Lund, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature Jörgen Toftered Date 2004-11-16

DOKUMENTDATABLAD enl SIS 61 41 21

List of papers

This thesis summarises the following papers which are referred to in the text by the roman numerals I-IV. Paper I and II are reprinted with kind permission of the publisher.

I: S. Bhattacharyya, J. Toftered and U.J. Nilsson

Synthesis of Rigid Polycyclic Secondary Diamines: Bis-(2,3:6,7-Iminodimethylene)anthracene and Bis-(2,3:6,7-Iminodimethylene)-9,10-dicarboxyethenoanthracene.

SYNLETT, **2003**, 1361-1363.

II: J. Toftered and U.J. Nilsson

Synthesis of Chiral Macrocycles by Cyclodimerization of Diamines with Stepwise Nucleophilic Aromatic Substitution of 1,5-Difluoro-2,4-dinitrobenzene.

SYNLETT, **2004**, 2517-2520.

III: J. Toftered and U.J. Nilsson

Reactivity fine-tuning of phenyl carbamates for efficient synthesis of amphiphilic macrocyclic bis-ureas and bis-thioureas.

in manuscript.

IV: J. Toftered and U.J. Nilsson

Synthesis of chiral macrocycles incorporating two different aromatic diamines.

in manuscript.

Abbreviations

9-BBN	9-Borabicyclo[3.3.1]nonane
AA	Amino acid
Ac	Acetyl
Bn	Benzyl
Boc	*tert*-butoxycarbonyl
Bz	Benzoyl
d	Distance
DCC	*N,N´*-Dicyclohexylcarbodiimide
DIBAL-H	Diisobutylaluminium hydride
DIC	*N,N´*-Diisopropylcarbodiimide
DMAc	Dimethyl acetamide
DMAP	4-Dimethylaminopyridine
DMF	Dimethyl formamide
DMS	Dimethyl sulphide
DMSO	Dimethyl sulphoxide
EDC	1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
e.g.	For example (exempli gratia)
EI	Electron impact
ES	Electrospray
Et	Ethyl
EWG	Electron withdrawing group
FAB	Fast atom bombardment
Fmoc	9-Fluorenylmethoxycarbonyl
HABA	2-(4-Hydroxyphenylazo)benzoic acid
HBTU	*O*-(Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry

i.e.	That is (id est)
i-Pr	Isopropyl
k	Rate constant
K_a	Association constant
LG	Leaving group
MALDI	Matrix assisted laser desorption/ionisation
Me	Methyl
MMFF94s	Merck molecular force field 1994 static
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
Nu	Nucleophile
PG	Protective group
PyBroP	Bromotri(pyrrolidino)phosphonium hexafluorophosphate
Red	Reduction
SEM	[2-(Trimethylsilyl)ethoxy]methyl
t-Bu	*tert*-butyl
TFA	Trifluoroacetic acid
TFFH	Tetramethylfluoroformamidinium hexafluorophosphate
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TOF	Time of flight
UV	Ultraviolet
Z	Benzyloxycarbonyl
Å	Ångström

Table of contents

LIST OF PAPERS **6**

ABBREVIATIONS **7**

TABLE OF CONTENTS **9**

1: BACKGROUND **11**

2: SYNTHESIS OF BUILDING BLOCKS **17**

SYNTHESIS OF DIIMIDE 18
REDUCTION OF DIIMIDE 18
ACYLATION OF DIAMINE 22
PROTECTIVE SCHEME - CHOICE AND REMOVAL 24
OTHER CHEMISTRY 25

3: NITROANILINES **27**

INTRODUCTION TO DIFLUORODINITROBENZENE 27
PRELIMINARY EXPERIMENTAL AND THEORETICAL CONSIDERATIONS 28
SYNTHESIS OF MACROCYCLES 35
PROTECTIVE GROUP CHEMISTRY 37

4: UREAS AND THIOUREAS **41**

INTRODUCTION TO UREA CROSS-LINKERS 43
ARYL CHLOROFORMATE AS CROSS-LINKING AGENT 44
SYNTHESIS OF ACTIVE CARBAMATES 46
SYNTHESIS OF UREAS - EVALUATION OF ACTIVE CARBAMATES 48
PROTECTIVE GROUP CHEMISTRY 53

5: MIXED DIAMINE MACROCYCLES **55**

DIFFERENT DIAMINES 55
FLEXIBLE MIXED MACROCYCLE 57
RIGID MIXED MACROCYCLE 59
CONCLUSION 62

6: CONCLUDING REMARKS **63**

7: ACKNOWLEDGEMENTS **71**

8: SUPPLEMENTARY DATA **73**

SUMMARY OF CONFORMERS AND CONFORMATIONAL ANALYSES 84

9: REFERENCES **85**

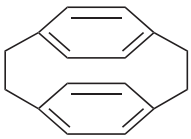
1: Background

Molecular recognition, *i.e.* the selective binding of a guest molecule to a host molecule, is a fundamental component of biological processes studied in life sciences.[1,2] By thoroughly studying molecular recognition, an extended knowledge and understanding of such biological processes, and perhaps even of life itself, may be obtained. While the answer of the philosophical question "What is the meaning of life?" is beyond the scope of this thesis,[3] the scientific endeavours documented in the following pages were all performed with the purpose of studying molecular recognition in biomimetic systems using the tools of organic chemistry.

Molecular recognition may be defined in terms of host-and-guest molecules as well as receptor-and-ligand molecules. The concept of a receptor is however not unambiguous in molecular recognition as the definition of the word "receptor" implies some sort of response upon binding of a specific ligand.[4] This definition excludes the possibility of a host molecule specifically recognising and binding a guest molecule without actually producing a response. Therefore, the term "receptor" should be avoided in projects evolving around molecular recognition until a physicochemical response has been observed upon binding of a specific guest to the host molecule.

The traditional approach towards investigation of molecular recognition in biological systems is to expose a specific receptor to a multitude of different ligands and observe differences in response from the receptor.[5] By comparing the chemical structures of the ligands with the degree of response from the receptor, extended knowledge about the molecular interaction between ligand and receptor may be obtained. One of the first examples of such a study was published in the year 1894 by the paramount chemist Emil Fischer[6] where he investigated the fermentation of different sugars with beer yeast[7] and introduced the "lock-and-key"-principle to the scientific community.[8,9] The "lock-and-key"-principle, *i.e.* where a receptor may only respond upon binding of a ligand as defined by the structure of the receptor, has been modified during the years with the induced fit theory,[1,10] where the receptor may adapt its structure to the ligand. Nowadays, the concept of molecular recognition is often visualised as a glove fitting a hand perfectly despite, or owing to, the flexibility of both the receptor (glove) and ligand (hand).

In the last decades, an alternative approach towards investigating molecular recognition in biological systems has become increasingly popular.[11-13] This approach works the opposite way around and is performed by studying the interactions between a multitude of host molecules and a specific guest molecule known to participate in biological processes. Ideally, the host molecule should be designed based on the chemical structure of the presumptive guest with ample opportunites of intermolecular bindings between host and guest. The host molecule should then be designed to allow for a multitude of both non-polar and polar interactions to assure the binding of a guest molecule.[14] These positions of interaction should preferentially be presented to the presumptive guest in a preorganised host to facilitate the formation of a host-guest complex without extensive conformational rearrangement.[1] For this purpose, macrocycles are useful as they consist of smaller segments attached in a large ring,[4] thus giving some degree of conformational preorganisation. As biological processes almost exclusively occur in an aqueous environment the host molecule should also be designed to have a reasonable solubility in water. Consequently, the host molecule should preferentially be an amphiphilic macrocycle incorporating both hydrophobic components, such as aromatic ring systems, and hydrophilic components, such as hydrophilic amino acids.



1

Figure 1-1: Cyclophane.

One popular class of compounds for application as host molecules is the class of macrocyclic compounds known as cyclophanes. Originally, this classification was applied to compounds with two *p*-phenylene units held together face to face by saturated aliphatic bridges, compounds such as structure **1** (Figure 1-1). Nowadays, this term has been widened to include macrocylic compounds incorporating aromatic ring systems and saturated or unsaturated bridges as alternating components of the ring structure.[4] Cyclophanes have received widespread attention in organic chemistry as they are challenging targets for synthesis and have interesting physical

properties.[15,16] Cyclophanes have also been used as intermediates in the total synthesis of complex natural products.[17] In the last few decades much of the research on cyclophanes has been focused on the inclusion of guest molecules into the cavity of the cyclophane.[18]

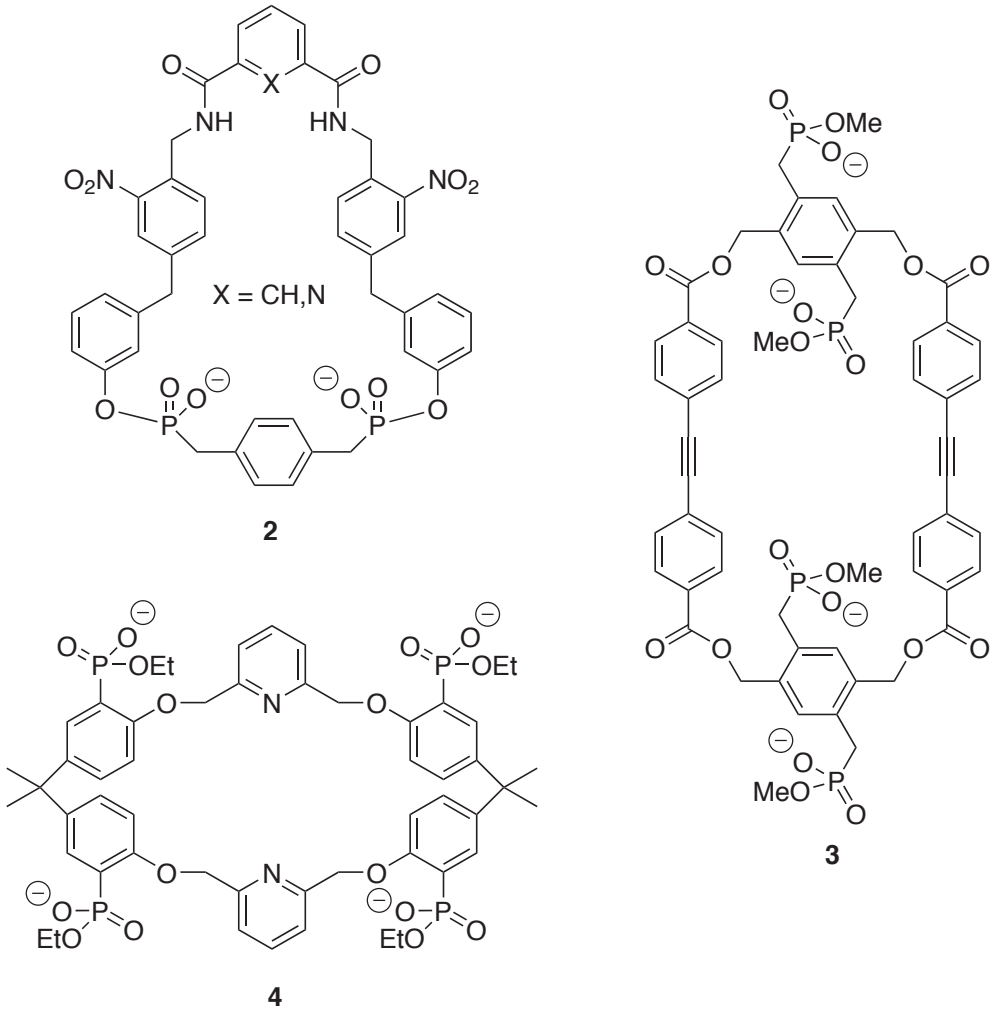


Figure 1-2: Recently reported host molecules. **2a**: X = CH, **2b**: X = N.

Some recent examples of cyclophanes as hosts for smaller guest molecules in aqueous or near-aqueous solution, *i.e.* a 1/1-mixture of CH_3OH/H_2O, are the structures **2**-**4** (Figure 1-2). Structure **2a** formed 1:1-complexes in near-aqueous solution with catecholamines such as noradrenaline and dopamine with binding constants of approximately 220 M^{-1}, whereas structure **2b** gave binding constants of approximately 140 M^{-1} with the same guests.[19] Cyclophane **3** was shown to form 2:1-complexes, *i.e.* two guest molecules and one host molecule, with a range of catecholamine-related guests in aqueous solution,[20] *e.g.* the antihypertensive β-

adrenergic blocker alprenolol[5] gave an overall binding constant $K_{a(2:1)}$ of $4.9*10^7$ M^{-2}. Host **4** formed a 1:1-complex with the methyl ester of amino acid lysine in water with a binding constant of 1200 M^{-1}. Methyl esters of other basic amino acids, namely histidine, ornithine and arginine, formed 2:1-complexes with the host **4**.[21]

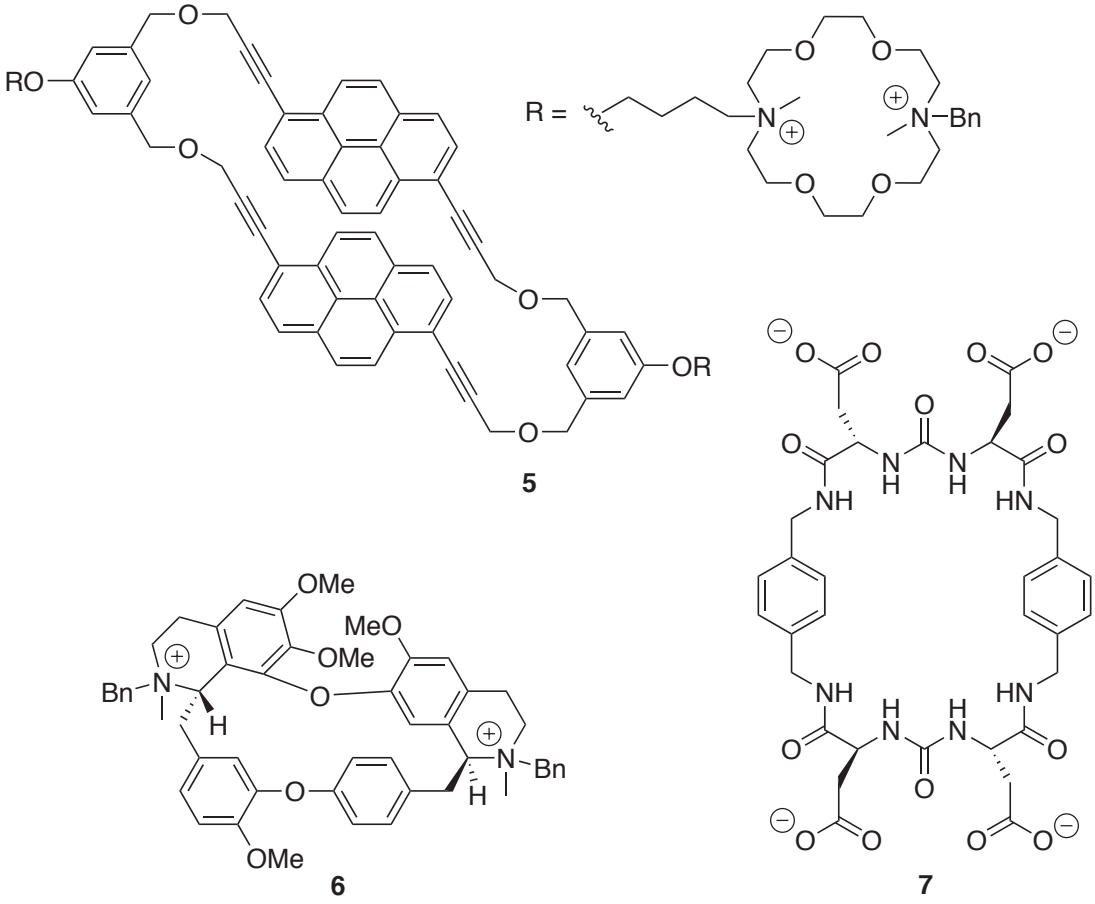
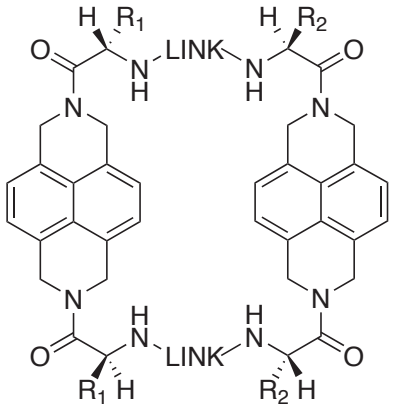


Figure 1-3: Other recent host molecules.

A more spectacular host can be found in structure **5** (Figure 1-3). This cyclophane was shown to form 1:1-complexes with different nucleotides in water with a preference for triphosphate nucleotides.[22] A binding constant of $1.3*10^6$ M^{-1} was observed with guanosine triphosphate, whereas the closely related guanosine diphosphate gave a binding constant of $4.0*10^3$ M^{-1}. The semisynthetic cyclophane **6**, derived from the natural product tetrandrine,[23] gave a more modest binding constant of 110 M^{-1} to adenosine triphosphate in water.

Recently, our laboratory has reported the synthesis of some cyclophanes based on *p*-xylylenediamine, cyclophanes such as **7**.[24] These substances have unfortunately shown poor performance as hosts in binding studies, presumably due to their large

conformational flexibility. This flexibility would be significantly lowered by substituting the xylylenediamines with more rigid diamines.

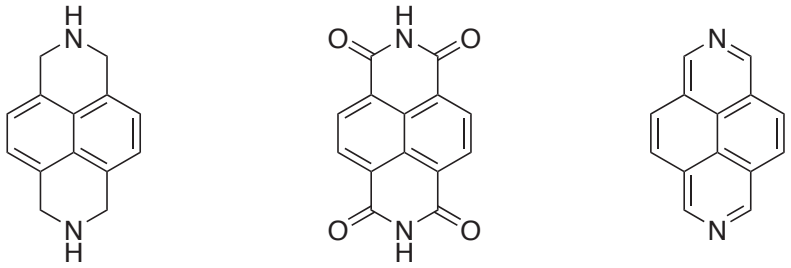


8

Figure 1-4: Potential host molecule.

The structure **8** should provide a more conformationally rigid cyclophane than **7** and could be a promising addition to the range of cyclophanes already reported (Figure 1-4). This cyclophane incorporates both hydrophobic aromatic ring systems and hydrophilic amino acids conjugated by an arbitrary linker. As the amino acids on the left side of the linker (R_1) may be different than the amino acids on the right side of the linker (R_2), and the linker itself is variable, the structure **8** may be viewed as a schematic presentation of a diverse range of cyclophanes.

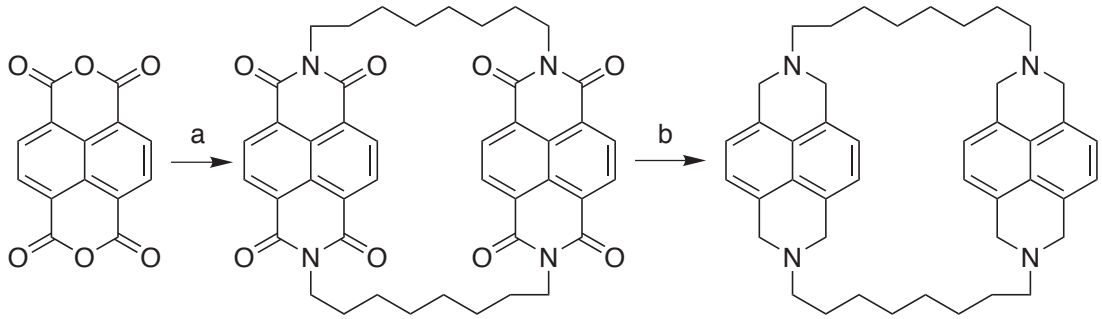
The cyclophane **8** may also be viewed as a derivative of 2,7-diaza-1,2,3,6,7,8-hexahydropyrene **9** (Figure 1-5). There is surprisingly little material published on the synthesis, characterisation and derivatisation of **9** in chemistry literature. The related compounds 1,4,5,8-naphthalenetetracarboxylic diimide **10** and 2,7-diazapyrene **11** are, on the contrary, well established as building blocks of different supramolecular structures.[25,26]



9 **10** **11**

Figure 1-5: Components of supramolecular systems.

It should be noted that a largely simplified version of macrocycle **8** already has been reported (Scheme 1-1).[27] This synthesis was perfomed by treating dianhydride **12** with 1,8-diaminooctane to give macrocycle **13**, which was then reduced into **14**. The macrocycle **13** was shown by chrystallography to form an inclusion complex with nitrobenzene where the guest nitrobenzene was located inbetween the two aromatic diimides. Macrocycle **14** was shown by NMR to form stable complexes with aromatic dicarboxylates such as 1,4-benzene dicarboxylate and 2,6-naphthalene dicarboxylate.



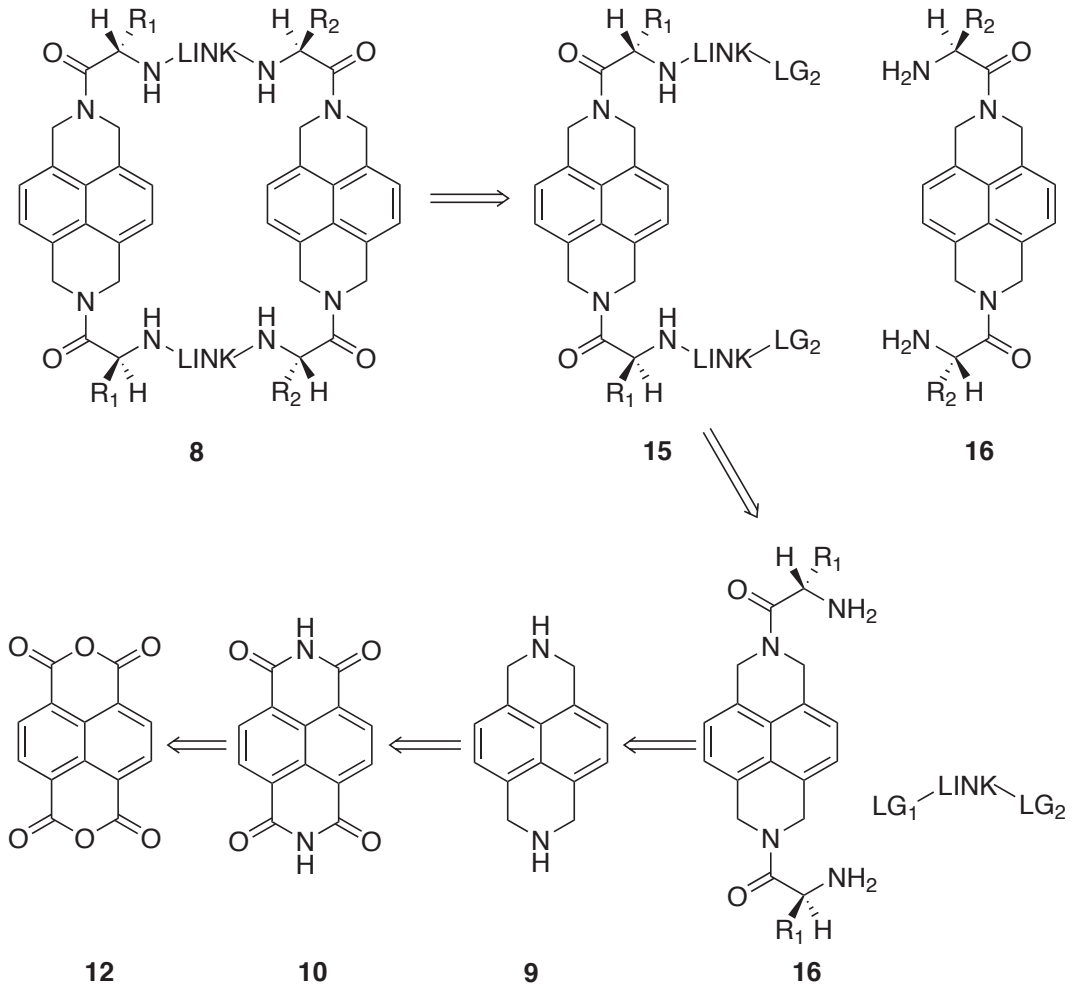
12 **13** **14**

Scheme 1-1: Synthesis of macrocycle **14**. a: 1,8-diaminooctane, DMF, 110-120°C. b: AlH_3, THF, 40°C.

With these findings at hand, the macrocycle **8** seemed as a most promising potential host for mono- or bicyclic amphiphilic guests such as carbohydrates, aromatic amino acids and neurotransmittors, as well as other interesting compounds such as drugs, stimulants and illegal substances. As the diamine **9** was largely overlooked in organic chemistry, the synthesis of cyclophane **8** could also be expected to include chemical pioneering and method developments.

2: Synthesis of building blocks

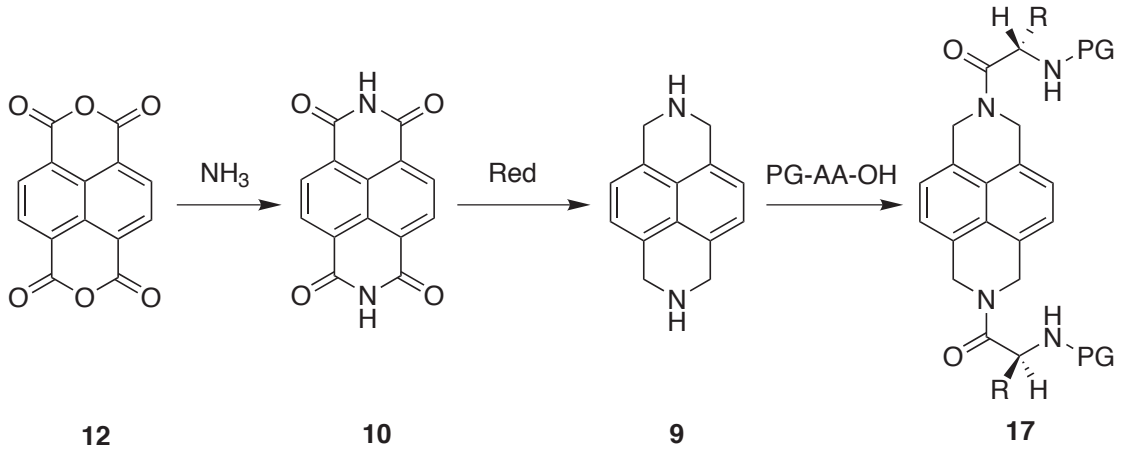
Retrosynthetic analysis (Scheme 2-1) of macrocycle **8** suggests that it is formed by condensation of fragment **15** and **16**, where **15** is available from another unit of **16** and a dielectrophilic linker. Fragment **16** may be further disconnected into diamine **9** which may be derived from commercially available tetracarboxylic dianhydride **12** via tetracarboxylic diimide **10**.



Scheme 2-1: Retrosynthesis of macrocycle **8**.

This chapter describes the synthesis of fragment **16** which is outlined in the scheme 2-2. Dianhydride **12** is treated with ammonia, or a derivative thereof, to produce diimide **10**. Diamine **9** is available by reduction of **10** and acylation of **9** with amino

acids yields substance **17**, which corresponds to fragment **16** in the retrosynthetic scheme above.



Scheme 2-2: Synthesis of building blocks.

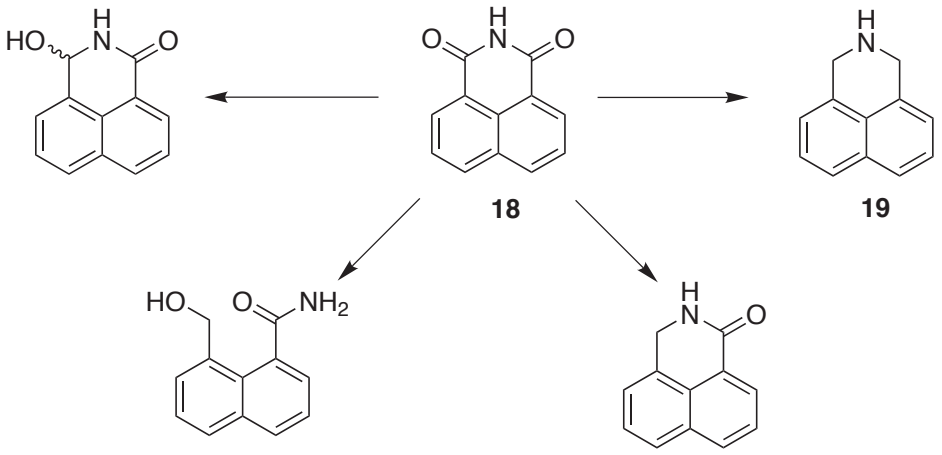
Synthesis of diimide

The synthesis of 1,4,5,8-naphthalenetetracarboxylic diimide **10** is easily accomplished by treating 1,4,5,8-naphthalenetetracarboxylic dianhydride **12** with aqueous ammonia. The first published synthesis[28] involved heating to increase the rate of reaction, a later modification of this procedure[29] recommended ambient temperature to obtain a better purity of **10**. Alternatively, **12** may be treated with formamide at elevated temperature to produce **10**.[30,31] Other approaches towards unsubstituted cyclic imides involves heating an anhydride or diacid with urea,[32] or treating a diacid with trifluoroacetamide and peptide coupling reagents.[33]

Reduction of diimide

The reduction of an imide is a fairly versatile transformation (Scheme 2-3). Apart from generating secondary amines, imide reduction may be used to prepare hydroxy lactams and amide alcohols,[34] as well as lactams.[35] Relatively few reagents have been used for reduction of aromatic imides into secondary amines. The reagents most commonly used are lithium aluminium hydride, aluminium hydride and borane. At the beginning of this project, there was no published procedure for reduction of **10** into 2,7-diaza-1,2,3,6,7,8-hexahydropyrene **9**. A number of reagents (Table 2-1) were

therefore evaluated in the reduction of commercially available 1,8-naphthaleneimide **18** into 2,3-dihydro-1H-benzo[de]isoquinoline **19**.



Scheme 2-3: Versatile reduction of imide **18**.

Table 2-1: Reduction of **18**.

Reducing agent	Yield of **19** (%)
BH_3•$HN(CH_3)_2$	0
$Na[AlH_2(OCH_2CH_2OCH_3)_2]$	10
9-BBN	0
KBH_4	0
$LiAlH_4$	0
DIBAL-H	10
BH_3•THF	65

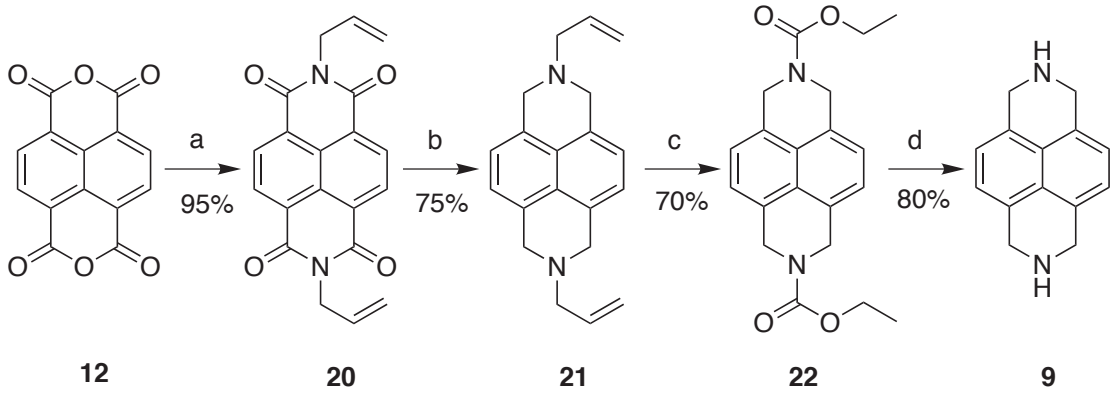
The most promising reagent BH_3•THF was then employed in the reduction of **10** into **9** where the yield dramatically decreased to 25%. Several other reagents, most of them different borohydrides, were tried under different reaction conditions without any significant improvement (Table 2-2).

Table 2-2: Reduction of **10**.

Reducing agent	Yield of **9** (%)
BH_3•THF	25
DIBAL-H	0
$LiBH_3N(i\text{-}Pr)_2$	20
$NaBH_3O_2CCF_3$	15
$NaBH_3OAc$	0
$NaBH_3OBz$	0
$LiBH_3OAc$	0
$LiBH_3OBz$	0
$NaBH_4$	0
AlH_3	0
Na, EtOH	0

The poor yield of **9** was at this moment assumed to originate from the low solubility of **10** in organic solvents, which was observed as early as 1887:[28] "Das Imid ist sehr schwer löslich in Alkohol, Aether, Eisessig, Benzol und Aceton." This quote may be roughly translated to: "The imide is very poorly soluble in alcohol, ether, acetic acid, benzene and acetone."

Substitution of the N-H-bonds of **10** with protective groups was investigated to obtain a diimide with better solubility. Attempts to directly alkylate **10** with BnCl or SEM-Cl were unsuccessful, which called for a new synthesis of *N*-alkylated diimide starting from **12** (Scheme 2-4).[36]

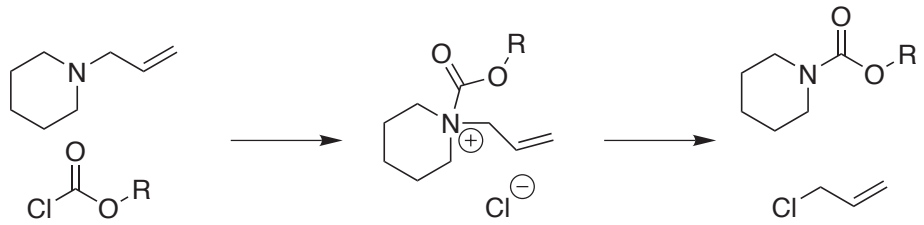


Scheme 2-4: Protective group approach to **9**. a: allylamine, H_2O, rt. b: AlH_3, THF, reflux. c: ethyl chloroformate, THF, reflux. d: KOH, H_2O, dioxane, reflux.

N,N´-Bis-allyldiimide **20** was easily obtained from **12**,[37] further reduction with aluminium hydride proceeded smoothly giving far better yields of diamine **21** than the ones hitherto obtained in reduction of **10** to **9**. Several synthetic methods are available for dealkylation of tertiary amines into secondary amines.[38] Deallylation is normally performed with base-promoted or transition metal-catalysed isomerisation into an enamine followed by hydrolysis. Attempts to deallylate **21** with potassium *t*-butoxide in DMSO[39] yielded only a dark material with poor solubility; no traces of **9** could be found. Transition metal-catalysed isomerisation with palladium on carbon or rhodium acetate did yield small amounts of **9** contaminated with unreacted starting material, intermediate mono-deallylated diamine and 2,7-diazapyrene.

An alternative approach for deallylation consists of acylative dealkylation into a carbamate followed by solvolysis into the desired product. By treating a tertiary amine with chloroformate, an *N*-dialkylated carbamate is produced (Scheme 2-5). 1-Chloroethyl chloroformate and ethyl chloroformate were investigated for this purpose

and the latter was found to give better yields of bis-carbamate. While chloroethyl carbamates are easily solvolysed with methanol,[40,41] ethyl carbamates require much harsher conditions for solvolysis. Refluxing **21** overnight in concentrated hydrochloric acid failed as not even trace amounts of deprotected **9** could be observed. Alkaline hydrolysis showed more promising results and this reaction was ultimately performed by refluxing **21** in a mixture of 2M potassium hydroxide and dioxane. The overall yield of **9** from **12** had now been increased from 24% to 40% by introduction of a protective group scheme.



Scheme 2-5: Acylative dealkylation with chloroformate.

Apart from the allyl group the protective groups tosyl, *t*-butyl and benzyl were also investigated. Treatment of **12** with tosylamide or *t*-butylamine failed to produce any diimide, benzylamine did however yield similar yields of diimide as observed with allylamine.[42] The reduction of dibenzylated diimide with aluminium hydride was equally successful, unfortunately the removal of the benzyl groups with ethyl or chloroethyl chloroformate did result in inferior yields of **9**.

The increased amounts of **9** available from the protective group approach served to a better characterisation of the solubility and purification procedure of **9**. A critical reinvestigation of the direct reduction of **10** to **9** showed that the poor yields obtained were largely due to an over-ambitious purification procedure including liquid-liquid extraction. When the crude diamine **9** was purified directly with column chromatography the yield was increased from 25% to 65%, thus making the protective group approach superfluous.

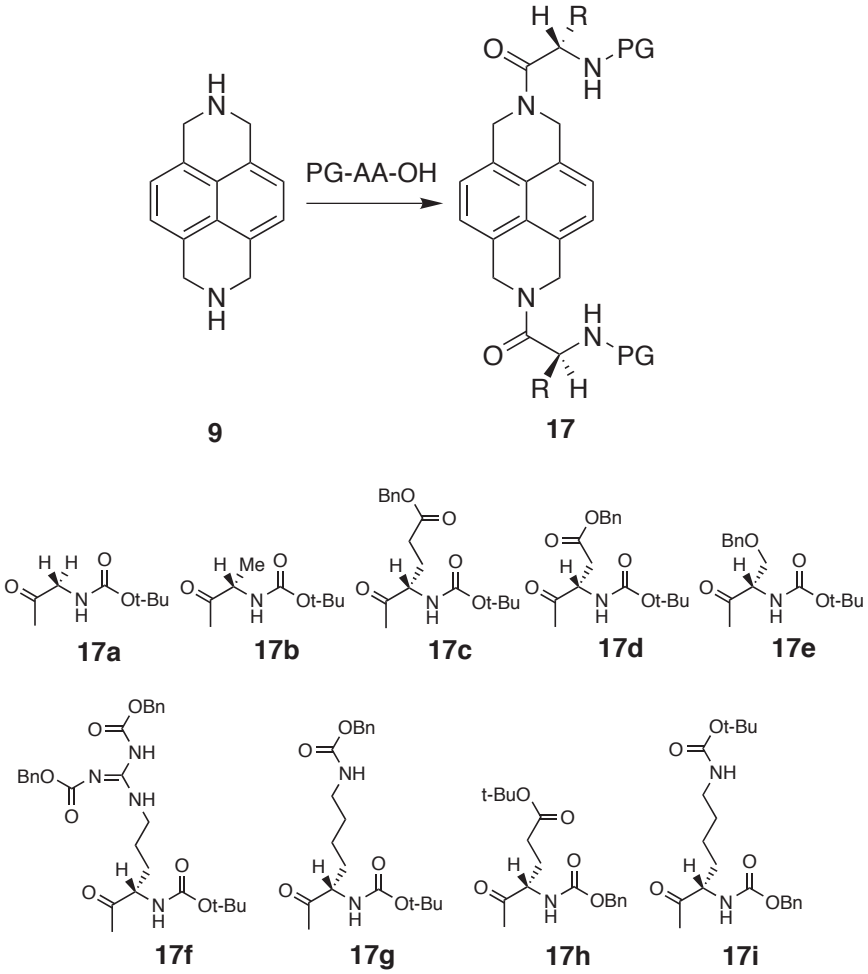
It should be noted that the reduction of **10** may be performed equally well with different borane reagents apart from BH_3•THF. The diamine **9** is obtained in similar yields with BH_3•DMS as well as borane generated *in situ* from sodium borohydride and boron trifluoride.[43] It should also be noted that diamine **9** is a slightly unstable compound and not suitable for prolonged storage. Compound **9** is obtained as a pale

solid which darkens upon contact with air or moisture, and subsequent reactions produce better yield with freshly prepared **9**.

Acylation of diamine

Acylation of a secondary amine with an amino acid is a reaction often encountered in organic synthesis and several coupling reagents have been evaluated for this purpose.[44] Various onium-type reagents (*e.g.* PyBroP and HBTU) and carbodiimides (*e.g.* DCC), as well as acid halogenating reagents (*e.g.* TFFH) are frequently employed in such acylations. The acylation of **9** was initially found to be difficult due to poor solubility of **9** in solvents commonly used in peptide couplings, solvents such as DMF and THF. NMR-analyses of **9** suspended in deuterated chloroform indicated that **9** was at least partially soluble in chloroform, suspending **9** in large volumes of chloroform (300 mL solvent / 1 g diamine) did indeed produce satisfactory yields of diacylated compounds **17** (Scheme 2-6, Table 2-3).

Commercially available chloroform is commonly stabilised with 1% ethanol and this stabilising agent must be removed from the chloroform prior to a coupling reaction.[45] If the reaction is performed in the presence of ethanol, large amounts of amino acid ethyl ester are formed as byproduct. One particular experiment resulted in a 91% yield of amino acid ethyl ester calculated from the amount of amino acid, the same experiment yielded 2% of the desired product **17**. Acylation of **9** with amino acids proceeds equally well with larger volumes of dichloromethane as solvent (370 mL solvent / 1 g diamine).



Scheme 2-6: Acylation of **9** with amino acids.

Table 2-3: Acylation of **9** with amino acids.

Entry	Amino acid	Protective scheme	Yield(%)
17a	Glycine	Boc-Gly-OH	83
17b	Alanine	Boc-Ala-OH	67
17c	Glutamic acid	Boc-Glu(OBn)-OH	72
17d	Aspartic acid	Boc-Asp(OBn)-OH	78
17e	Serine	Boc-Ser(OBn)-OH	72
17f	Arginine	Boc-Arg(Z_2)-OH	36[a]
17g	Lysine	Boc-Lys(Z)-OH	57
17h	Glutamic acid	Z-Glu(Ot-Bu)-OH	62
17i	Lysine	Z-Lys(Boc)-OH	12[b]

a: old diamine. *b*: DMAP substituted with pyridine.

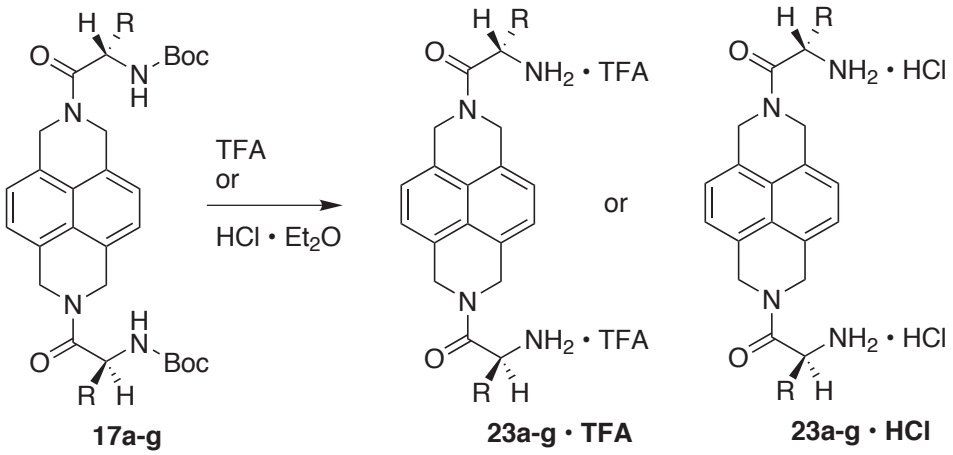
The coupling was typically performed with EDC/HOBt/DMAP as these reagents are easily separated from **17** by chromatography or precipitation. EDC may be substituted with DIC in reactions where **9** is acylated with amino acids incorporating long lipophilic side chains (*e.g.* entries **17c-e** in Table 2-3). Amino acids with short or

non-existent side chains, such as alanine or glycine, result in bis-acylated compounds **17a-b** with poor solubility. These compounds tend to precipitate together with the diisopropylurea formed during acylation of **9** with DIC. The relatively poor yield of **17f** is due to a reaction performed with substandard purity of **9**, whereas the inferior yield of **17i** is dependent upon a reaction where DMAP was substituted with pyridine.

Protective scheme - choice and removal

The synthesis of **17** from **9** was mostly performed with amino acids containing a Boc-protected amino group. Various incarnations of a benzyl moiety were used as side chain protective groups in the case of amino acids with hydrophilic functionalities in the side chain, amino acids such as glutamic acid.

This combination of protective groups is commonly referred to as the Boc/Bn strategy[46] and relies on the different acid sensitivity between Boc and Bn. Debenzylation via acidolysis normally requires strong mineral acids whereas Boc may be acidolysed with weaker acids such as TFA or dilute HCl•Et_2O. Repeated Boc-acidolysis in the synthesis of longer peptides may result in some debenzylated byproducts, however such byproducts are unlikely in the syntheses of this project. Removal of Boc (Scheme 2-7) from **17** with TFA or HCl•Et_2O did indeed produce quantitative yield of bis-ammonium trifluoroacetate or bis-ammonium chloride salts **23** with only one notable exception. The deprotection of arginine derivative **17f** with TFA gave an unexpectedly large removal of the side chain protective groups Z.



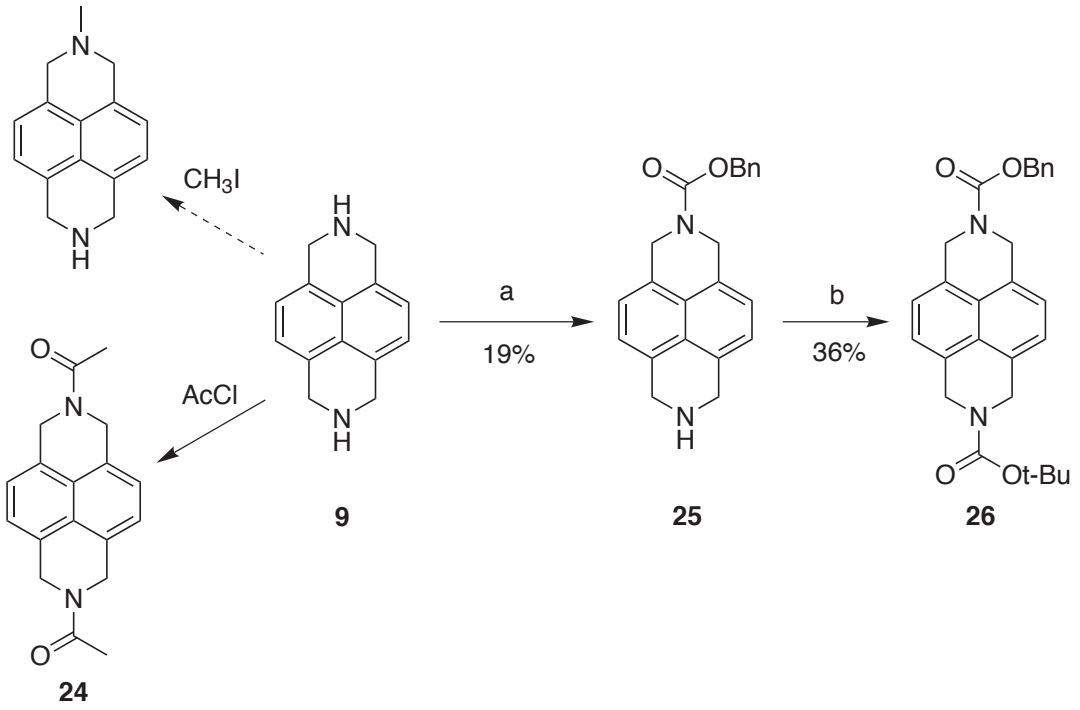
Scheme 2-7: Removal of the protective group Boc.

It should be noted that the counterion of the deprotected **23** has a significant impact on the solubility. Trifluoroacetate salts **23a-g•TFA** are readily soluble, whereas hydrochloride salts **23a-g•HCl** have poor solubility, in THF. It should also be noted that the deprotections were performed in the presence of triethylsilane which serves as a carbocation scavenger.[47] Omitting this scavenger yielded **23** contaminated with a dark polymeric material.

Apart from the Boc/Bn protective scheme, the Z/tBu and the Fmoc/tBu were also investigated. Acylation of **9** with Z/tBu protected amino acid proceeded smoothly to give **17h-i**, but the removal of Z by palladium catalysed hydrogenation with ammonium formate as hydrogen donor did not produce quantitative yields of **23**. Attempts to acylate **9** with Fmoc protected amino acids were unsuccessful, compound **9** reacted as an Fmoc-deprotecting base rather than as a nucleophile.

Other chemistry

The synthetic procedure described so far has been focused on symmetrical derivatisation of **9**. Some attempts of asymmetrical derivatisation were performed (Scheme 2-8).

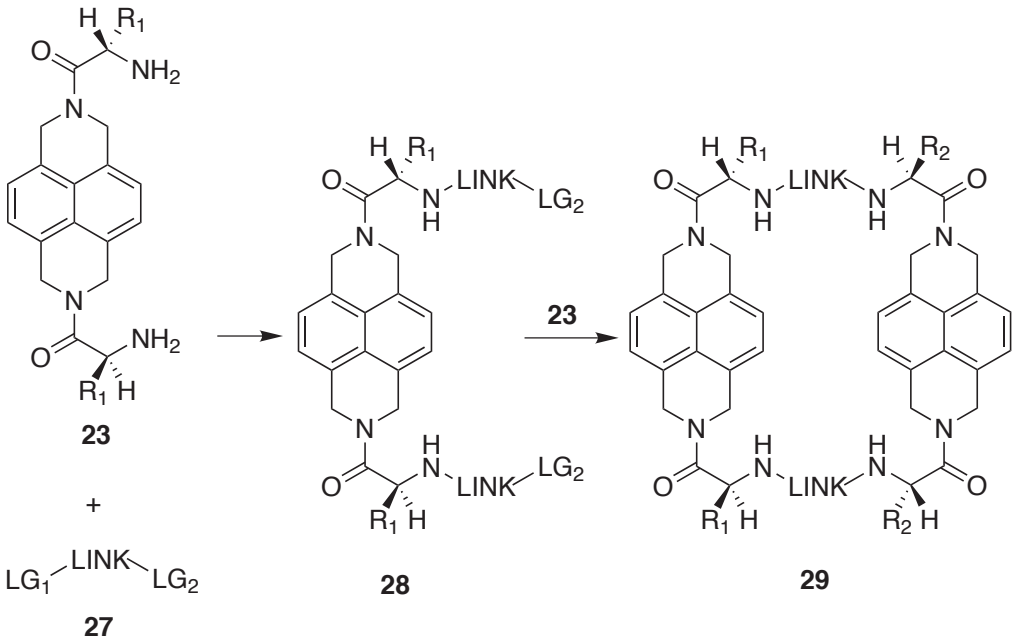


Scheme 2-8: Asymmetrical derivatisation of **9**. a: benzyl chloroformate, Cs_2CO_3, THF, CH_2Cl_2, rt. b: Boc_2O, DMAP, Cs_2CO_3, THF, rt.

Monoalkylation of **9** with methyl iodide was unsuccessful as the reaction produced a complicated mixture of monomethylated and dimethylated products together with unreacted **9**, no attempt of separation and purification was performed. Monoacylation with acetyl chloride was also unsatisfactory as the reaction only yielded diacetylated product **24**. Treatment of **9** with benzyl chloroformate did however result in some monoreacted **25**, the remaining amino functionality reacted with di-*t*-butyl dicarbonate to produce orthogonally protected diamine **26**. The yields of these two reactions are unoptimised, the latter reaction is likely to give at least twice as high yield of **26** with some modifications. Nevertheless, this procedure should be applicable to several other chloroformates and opens up the possibility of acylating **9** with two different amino acids.

3: Nitroanilines

Condensation of two fragments of **23** require some sort of dielectrophilic reagent with reactivity towards nitrogen nucleophiles (Scheme 3-1). The dielectrophile **27** should preferably have a discriminating reactivity towards monosubstitution to simplify isolation and purification of intermediate **28** while at the same time remain reactive after monosubstitution to enable synthesis of macrocycle **29** under reasonably mild reaction conditions. Furthermore, the linker **27** should include additional opportunities for intermolecular interactions between host **29** and guest molecules. The linker should also be fairly rigid to facilitate the formation of a conformationally defined binding pocket. Finally, the linker **27** itself and byproducts generated from **27**, *i.e.* leaving groups, should be easy to separate from the desired molecules **28** and **29**.



Scheme 3-1: General synthesis of macrocycles.

Introduction to difluorodinitrobenzene

The dielectrophile 1,5-difluoro-2,4-dinitrobenzene **30** (Figure 3-1) caught attention partly as a cross-linking reagent for proteins,[48] partly through the derivative **31** (also

known as Marfey´s reagent)[49] which is a chromophoric tool for determining the degree of racemization of amino acids during solid phase peptide synthesis.[50]

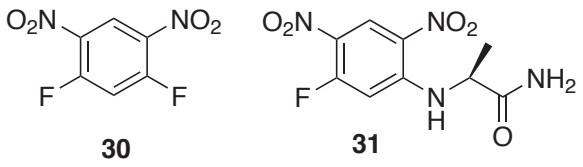


Figure 3-1: Cross-linking reagent and Marfey´s reagent.

Remarkably little material is published on the use of **30** in macrocyclisations, to this date **30** has only appeared in two different macrocyclic syntheses (Figure 3-2). The first synthesis[51] aimed at producing rigid tetranitro-resorcinarenes such as **32** whereas the second synthesis[52] targeted the cyclic peptide **33**. It is noteworthy that in neither one of these examples did **30** react with two nucleophilic nitrogens to produce a dinitrodianiline.

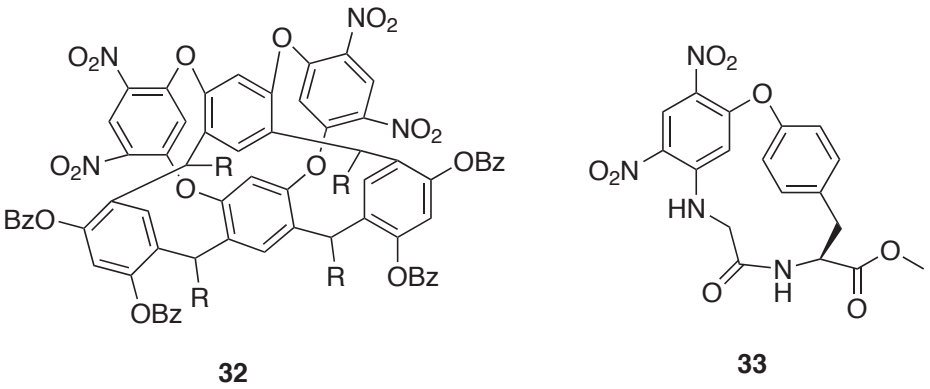


Figure 3-2: Macrocyclic compounds incorporating **30**.

Preliminary experimental and theoretical considerations

Conformational analyses were performed with a macrocycle **29** incorporating the linker **30** and serine residues **23e** with a methyl ether on the amino acid side chain. Monte Carlo simulations using the program MacroModel, based on the force field MMFF94s[53] with chloroform as solvent, yielded 182 different conformers with energies within 5 kcal/mol relative to the lowest energy conformer **C1**. The vast majority of these conformers were small variations of the cleft-like **C1** (Figure 3-3, top-left and top-right structure are two diffent perspectives of **C1**) with different

orientations of the amino acids´ side-chains or the dinitrodianiline moieties, though some spectacular exceptions were found. The exceptions were largely conformers **C2** and **C3** with three of the aromatic moieties stacked on top of each other in a "hamburger" fashion (Figure 3-3, bottom-left and bottom-right). While the conformers **C3** with a naphthalene moiety stacked inbetween two dinitrodianilines were of a relatively high energy (4.9 kcal/mol higher than **C1**), the conformers **C2** with a dinitrodianiline stacked inbetween two naphthalenes were quite close in energy relative to **C1**. These "hamburgers" started to appear amongst other conformers with an energy of 1.0 kcal/mol higher than **C1**.

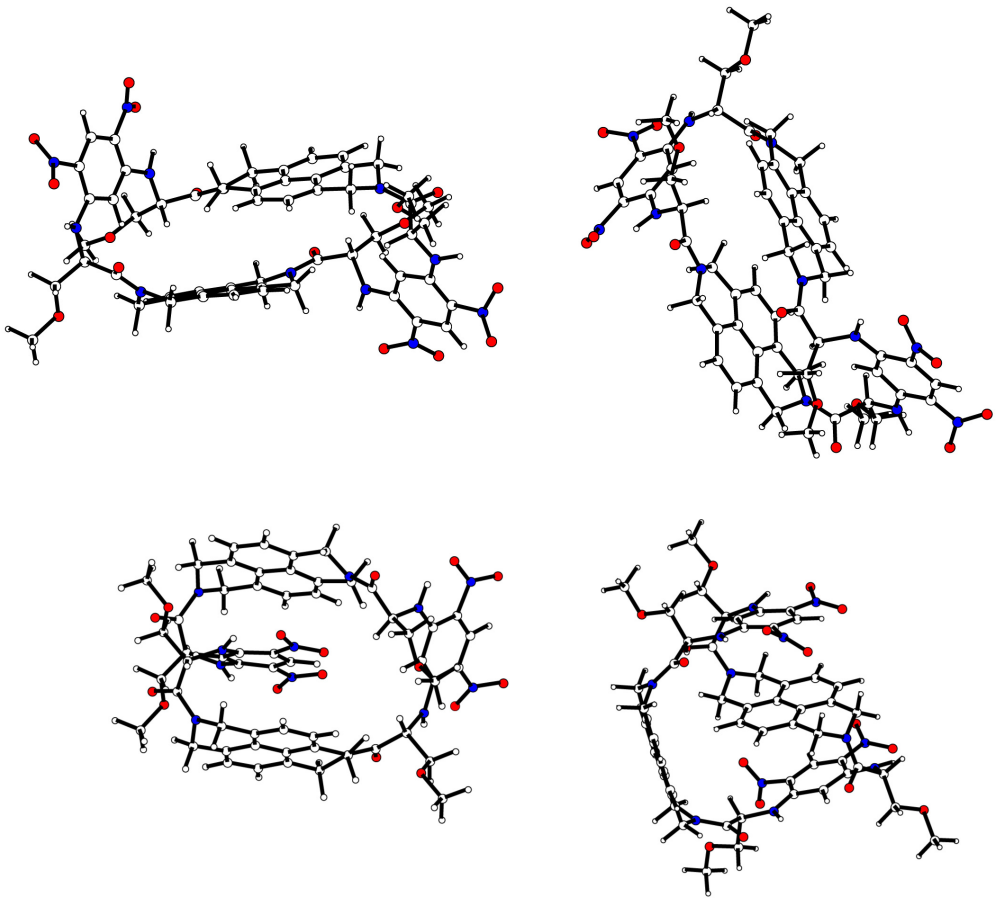


Figure 3-3: Representative conformers of **29**. Top-left and top-right: lowest energy conformer **C1**. Bottom-left and bottom right: two hamburger-conformers **C2** and **C3**.

The distance between the two aromatic diamines was measured at three different positions (Figure 3-4) in the conformers presented above, namely the distance between the two pairs of benzylic nitrogens (d_1 and d_2) as well as the distance between

the centroid position of the aromatic ring systems (d_3). The distances (Table 3-1) may appear peculiar, but these measurements are just a reflection of the tilted orientation of the two separate aromatic diamines.

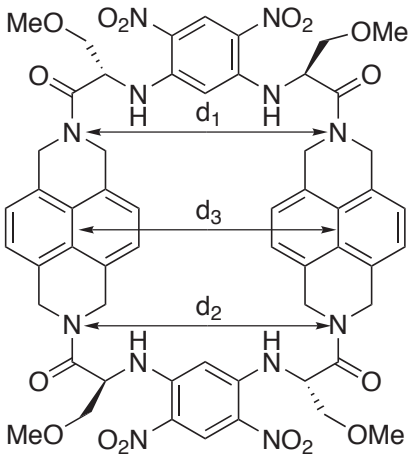
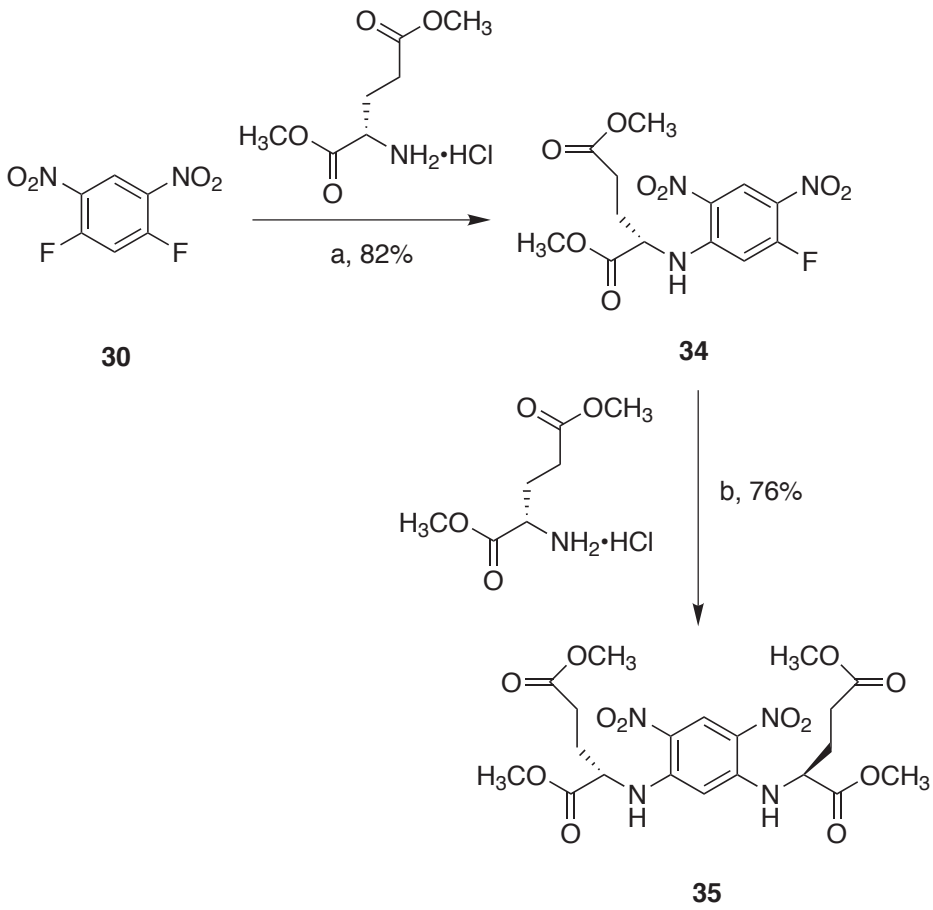


Figure 3-4: Distances measured in comformers.

Table 3-1: Measured distance between aromatic diamine moieties.

Conformer	d_1 (Å)	d_2 (Å)	d_3 (Å)
C1	6.0	6.3	5.6
C2	7.4	6.8	7.5
C3	6.2	7.6	6.5

Some preliminary experiments were performed to evaluate the performance of **30** as dielectrophile by sequential reaction with glutamic acid dimethyl ester (Scheme 3-2). The first substitution proceeded rapidly at room temperature and produced an acceptable yield of intermediate **34**. This intermediate was found to be quite stable as it could be stored for several days at room temperature without any significant decomposition. The second substitution required somewhat harsher conditions, the reaction proceeded sluggishly at room temperature and needed refluxing conditions overnight to produce **35**.



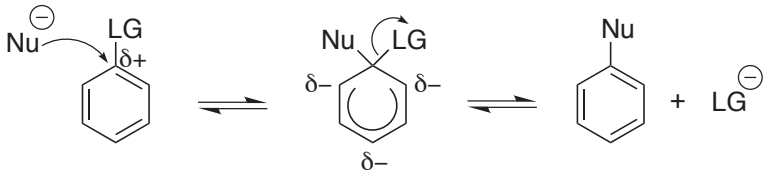
Scheme 3-2: a: $NaHCO_3$, THF, rt. b: $NaHCO_3$, THF, reflux.

The different reactivity of **30** and **34** in substitution with amino acid esters obviously follows the general trends in reactivity observed in nucleophilic aromatic substitutions with respect to activating and deactivating substituents.[54,55] Compound **30** contains two strongly activating nitro groups and two weakly activating fluorines. Compound **34** contains two strongly activating nitro groups, one weakly activating fluorine and one strongly deactivating amino substituent (Table 3-2).

Table 3-2: Activating and deactivating substitutents in nucleophilic aromatic substitution.

Activating groups in order of decreasing activating power	Deactivating groups in order of decreasing deactivating power
Diazonium salt group ($-N_2^+$)	Amino group (NH_2)
Cationic carbon ($-CR_2^+$)	Hydroxyl (OH)
Nitroso group (NO)	Dimethylamine ($(CH_3)_2N$)
Nitro group (NO_2)	Ethoxy (OCH_2CH_3)
Methylsulfonyl (CH_3SO_2)	Methoxy (OCH_3)
Trimethylammonium ($(CH_3)_3N^+$)	Methyl (CH_3)
Trifluoromethyl (CF_3)	*Tert*-butyl ($(CH_3)_3C$)
Acyl groups (RCO)	
Cyano (CN)	
Carboxylic acid (COOH)	
Ionized sulfo (SO_3^-)	
Halogens (Cl, Br, I, F)	
Carboxylate (COO^-)	
Phenyl	

The weakly activating power of fluorine is rather obscure; fluorine has been found to activate nucleophilic substitution in *ortho*- and *meta*-positions but deactivate substitutions in *para*-positions.[56] The activating effect of fluorine in *ortho*-position has been explained through the inductive effect of the strongly electronegative fluorine which lowers electron density at the carbon carrying the leaving group thus making that carbon more susceptible towards nucleophilic attack. Fluorine in *para*-position, on the other hand, destabilises the Meisenheimer complex **36** (also referred to as a σ-complex) through electron-pair repulsions.[57] This repulsion is obviously not apparent when fluorine is in *meta*-position (Scheme 3-3).



36

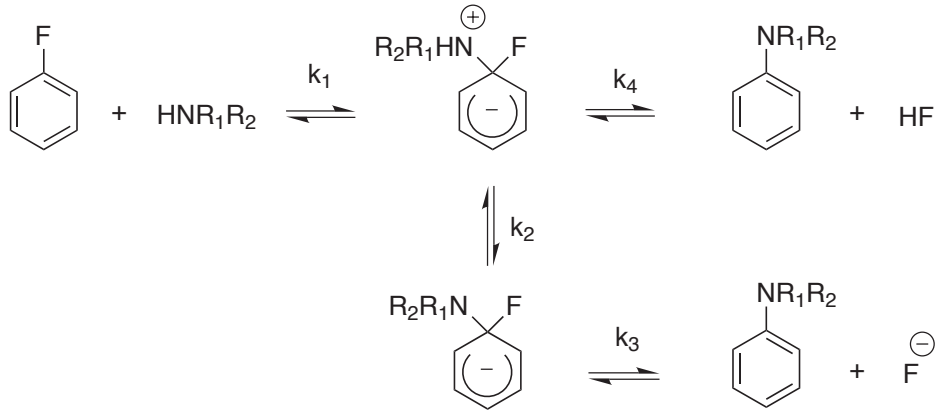
Scheme 3-3: Formation of Meisenheimer complex.

It is noteworthy that both the fluorine and the nitro group are considered as good leaving groups in nucleophilic aromatic substitutions (Table 3-3). Fluorine is generally the better leaving group in nucleophilic aromatic substitutions when a neutral amine is used as nucleophile, though certain exceptions exists.[58]

Table 3-3: Leaving groups in nucleophilic aromatic substitutions.

Leaving groups in approximate order of decreasing mobility
Fluorine
Nitro
Chlorine, bromine, iodine
Azido
Sulfonate groups (OSO_2R)
Ammonium groups (NR_3^+)
Phenoxy groups (OAr)
Alkoxy groups (OR)
Thioether groups (SR and SAr)
Sulfonyl groups (SO_2R)
Amino groups

The reaction mechanism during nucleophilic aromatic substitution is generally found to proceed through a Meisenheimer complex.[55,58] In the case of substituting fluorine with neutral primary or secondary amines the mechanism may be divided into three separate steps (Scheme 3-4): addition of nucleophile (k_1), deprotonation of zwitterionic intermediate (k_2) and expulsion of leaving group (k_3 and k_4).



Scheme 3-4: Mechanism of substitution with neutral amine as nucleophile.

The rate limiting step has been found to depend on both the nature of the nucleophile and the solvent used. Sterically hindered nucleophiles tend to give reactions where addition of nucleophile (k_1) is rate limiting.[59] Deprotonation of the zwitterionic intermediate (k_2) is rate limiting primarily when the reaction is performed in protic solvents.[60] Studies of fluoride kinetic isotope effects in the reaction between piperidine and 2,4-dinitrofluorobenzene have shown that expulsion of leaving group (k_3 and k_4) is rate limiting when the reaction is perfomed in THF, whereas addition of nucleophile (k_1) is rate limiting when the reaction is performed in acetonitrile. This

difference was explained by different acid-base properties of the two solvents THF and acetonitrile.[61] THF was used as a solvent in the synthesis of **34** from **30** and as the amino acid ester is a slighty less sterically hindered nucleophile than piperidine it is likely that expulsion of the leaving group is the rate limiting step in this reaction.

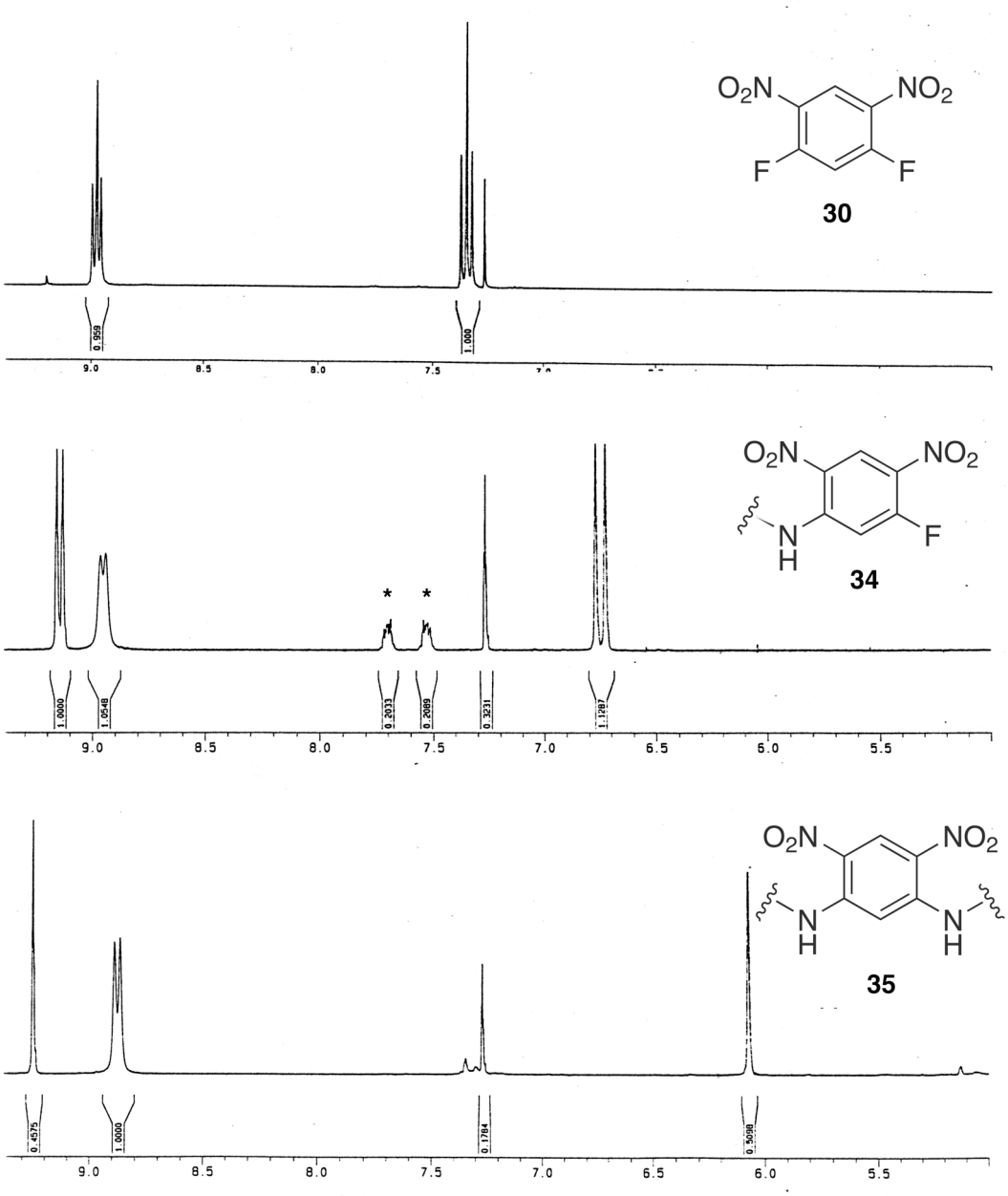


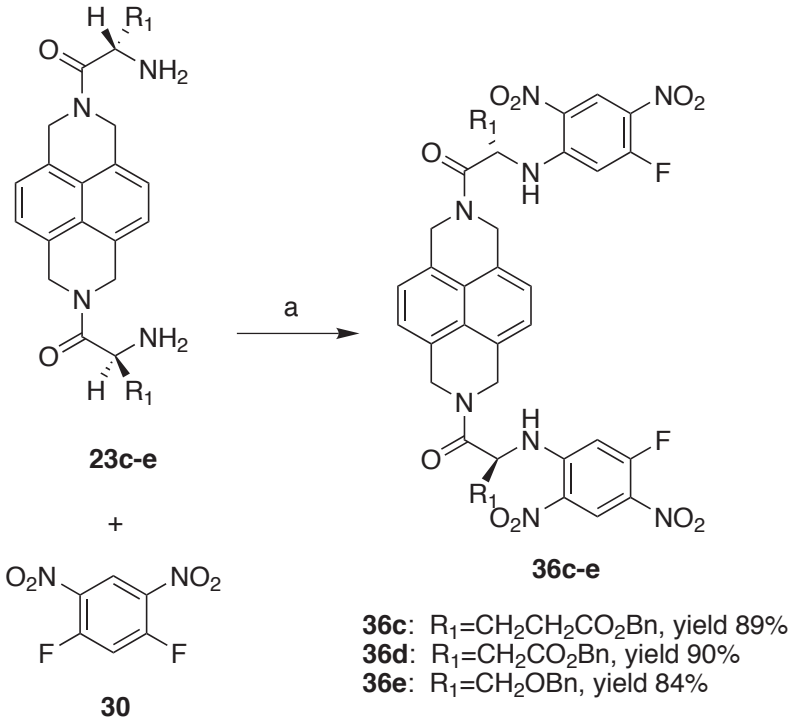
Figure 3-5: ^{1}H-NMR-spectra of **30**,**34** and **35**. *: Compound **34** is contaminated with a small amount of dioctyl phthalate.

The ^{1}H-NMR spectra of compounds **30**, **34** and **35** in deuterated chloroform contain a striking example of ^{1}H-^{19}F coupling (Figure 3-5).[62] The protons of unsubstituted **30** are observed as two separate triplets, these triplets are transformed

into duplets in **34** and singlets in **35** thus clearly indicating the expulsion of two separate fluorine atoms. This coupling pattern was obviously of interest in the use of **30** as a dielectrophilic linker in the synthesis of macrocycles.

Synthesis of macrocycles

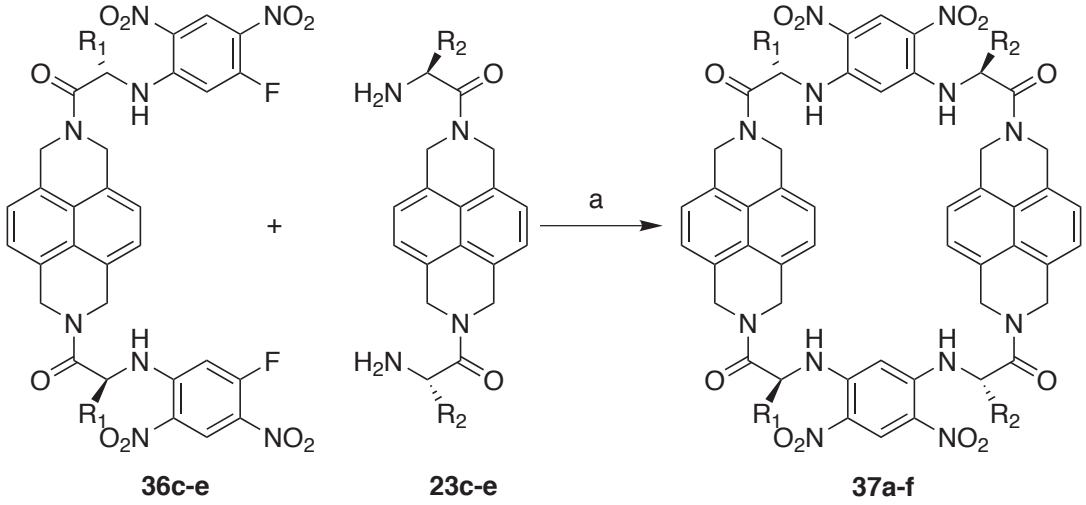
Diamines **23c-e•TFA** reacted smoothly with an excess of **30** at room temperature and produced the desired compounds **36c-e** in most satisfying yields (Scheme 3-5). The reaction was normally performed with caesium carbonate as a base, though the reaction proceeded equally well with sodium bicarbonate. ^{1}H-NMR analyses of the compounds **36c-e** clearly showed the desired ^{1}H-^{19}F coupling mentioned above (Figure 3-5), thus confirming the inclusion of linker molecule **30** at both nucleophilic nitrogens in diamines **23c-e**. Reaction was also attempted with diamines **23a-b•TFA** but these reactions failed to produce any compounds **36a-b**, presumably due to poor solubility of these compounds.



Scheme 3-5: First nucleophilic aromatic substitution. a: Cs_2CO_3, THF, rt.

Synthesis of macrocycles **37a-f** by condensation of fragments **36c-e** with **23c-e•TFA** did as expected require slightly harsher reaction conditions (Scheme 3-6).

Apart from lower reactivity of **36c-e** towards nucleophilic aromatic substitution the reaction was also performed under diluted conditions (also known as the Ruggli-Ziegler diluted principle)[63,64] to suppress polymerisation and favour formation of macrocylic product. Concentrations of **23c-e•TFA** and **36c-e** were normally in the range of 0.5-1.0 mM. These factors combined resulted in a sluggish rate of reaction when condensation was attempted at ambient temperature, trace amounts of **37a-f** were detected together with unreacted starting materials after several days.



Scheme 3-6: Second nucleophilic aromatic substitution. a: Cs_2CO_3, THF, reflux.

Table 3-4: Synthesis of macrocycles.

Reagent	Reagent	**Product**	R_1	R_2	Yield (%)
36e	**23e**	**37a**	CH_2OBn	CH_2OBn	22
36d	**23d**	**37b**	CH_2CO_2Bn	CH_2CO_2Bn	20
36c	**23c**	**37c**	$CH_2CH_2CO_2Bn$	$CH_2CH_2CO_2Bn$	(16)[a]
36e	**23d**	**37d**	CH_2OBn	CH_2CO_2Bn	(10)[a]
36e	**23c**	**37e**	CH_2OBn	$CH_2CH_2CO_2Bn$	18
36c	**23c**	**37f**	CH_2CO_2Bn	$CH_2CH_2CO_2Bn$	20

a: Substandard purity of product.

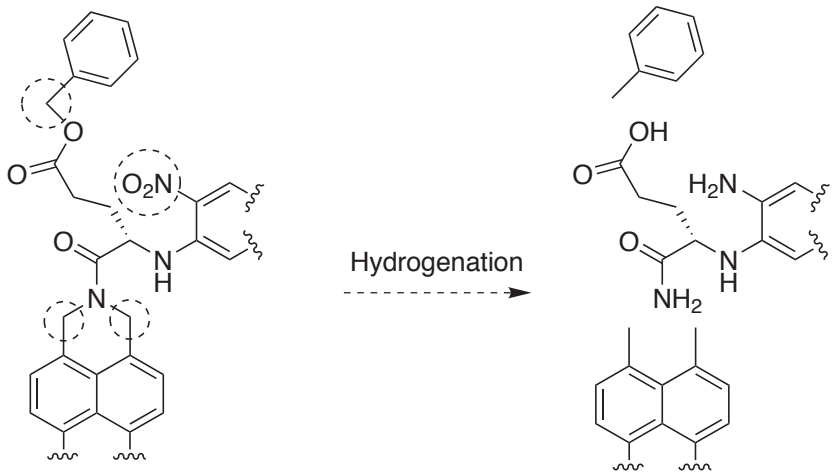
Performing the reaction under refluxing conditions resulted in reasonable yields of macrocycles **37a-f** (Table 3-4). Nucleophilic aromatic substitution of aryl halides with amines may be catalysed with CuI,[65] though this effect depends on the aryl halide in the order of I>Br>Cl>F. Thus substitution of aryl fluorides are least likely to be catalyzed with CuI. As expected, addition of CuI to the reaction mixture did not have any noticeable effect on reaction rate or yield of **37**. The combination of long reaction time and high temperature under basic conditions raised the issue of amino acid

racemisation, but other examples of nucleophilic aromatic substitution with amino acids[66] indicate that racemisation is of less concern even under more vigorous conditions (90°C, 48 h).

The identity and purity of compounds **37a-f** were unfortunately difficult to analyse with ^{1}H-NMR as **37a-f** only resulted in poorly resolved spectra with large broadening of peaks, presumably due to slow conformational equlibria within the macrocyclic compounds.[67] Similar macrocycles are known to yield ^{1}H-NMR-spectra with poor resolution.[68] Attempts to improve resolution by performing high-temperature analyses in deuterated DMSO were unsuccessful as compounds **37a-f** tend to decompose at elevated temperatures. On the other hand, ^{13}C-NMR analyses yielded spectra of **37a,b,e,f** where the fluorodinitroaniline moieties obviously had been substituted into the desired dinitrodianilines. Analytical reversed-phase HPLC using water-acetonitrile as eluent gave chromatograms for **37a,b,e,f** with one dominant peak corresponding to approximately 95% of the total peak area. Mass spectrometry using MALDI-TOF was unsuccessful as compounds **37a-f** (and **36c-e**) yielded spectra with a staggering amount of noise peaks, though FAB-HRMS experiments clearly confirmed the composition of substances **37a-f**.

Protective group chemistry

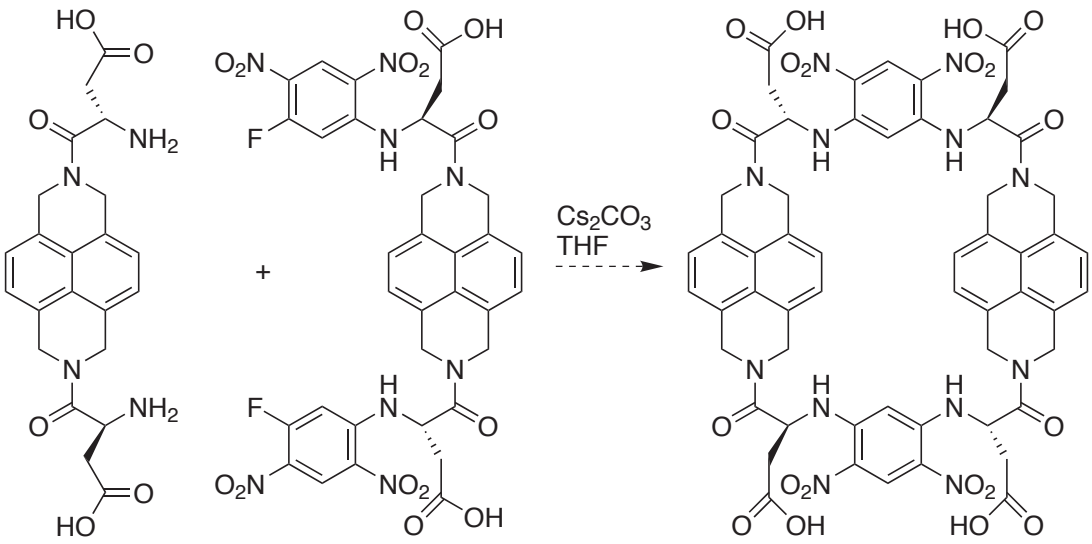
The macrocycles **37a-f** were readily soluble in halogenated solvents as chloroform or dichloromethane and, as might be expected, hardly soluble at all in aqueous solutions. By removing the benzyl protective groups the macrocycles **37a-f** should apparently become more polar and more soluble in an aqueous environment. Several methods are available for removing benzyl groups,[38] methods such as transition metal catalysed hydrogenation, acidolysis with strong acids and, in the case of benzyl esters, hydrolysis under acidic or alkaline conditions.



Scheme 3-7: Positions most susceptible to hydrogenation in **37**.

Hydrogenation with palladium on carbon and formic acid as hydrogen donor[69] yielded a most complicated mixture according to HPLC and MALDI-TOF. Apart from removal of benzyl groups, fragmentation and decomposition of the macrocycle was observed, presumably caused by hydrogenolysis of the benzylic positions in the diazahexahydropyrene moieties. Reduction of the nitro groups into anilines further complicated the reaction. No attempts on isolation and purification were performed with this mixture of products (Scheme 3-7).

Acidolysis with HBr in acetic acid yielded a slightly less complicated crude product though no fully deprotected macrocycle could be isolated from this material. Deprotection with TMS-I[70] was equally unsuccessful, as well as alkaline hydrolysis with KOH. All of these methods gave essentially the same result, namely disappearance of the fully protected starting material followed by appearance of several different products with poor solubility in most solvents except DMSO. Even though the products sometimes could be separated with HPLC the different products were unidentifiable by NMR, MALDI-TOF or FAB-MS.



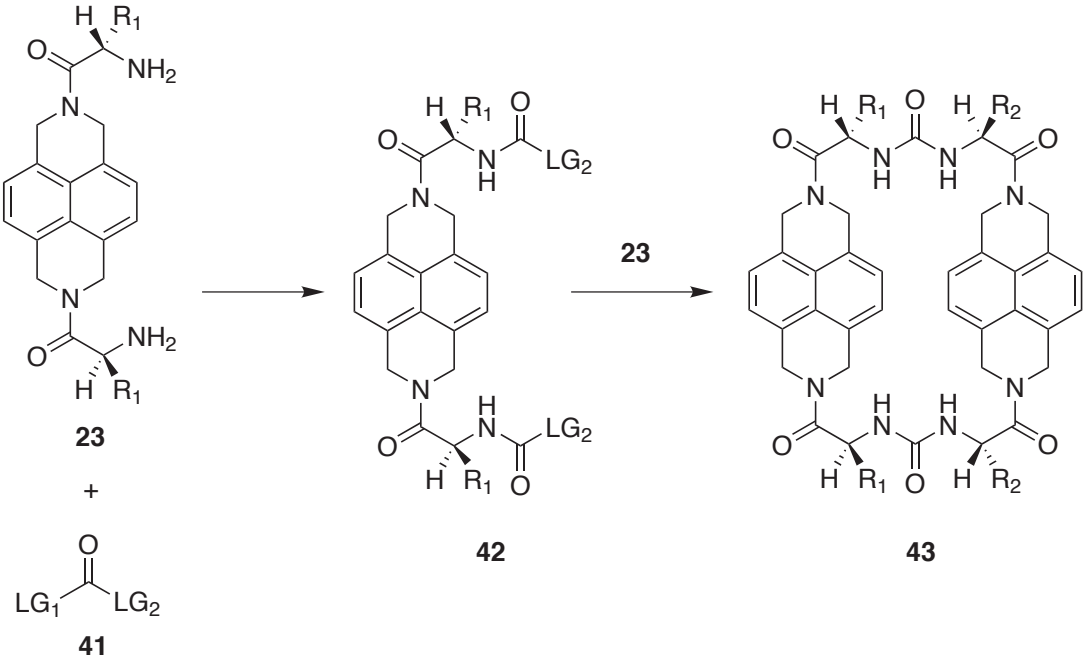
38 **39** **40**

Scheme 3-8: Macrocyclisation with deprotected starting materials.

To avoid the obviously difficult deprotection of macrocycles **37** a slightly different synthetic scheme was envisioned (Scheme 3-8). By performing the macrocyclisation with unprotected starting materials such as **38** and **39** the reaction could be expected to directly produce the desired target compound **40**. Unfortunately, this procedure is yet to succeed, as no compound **39** has been obtained despite several attempts.

4: Ureas and thioureas

The urea (sometimes referred to as the ureine) is a structural element often incorporated in macrocyclic compounds as well as in various peptide-related compounds. Together with its sulphur analogue thiourea, it provides a rigid linkage of two nitrogens giving a clearly defined pattern of hydrogen bondings frequently employed in molecular recognition.[71] It has been observed that the thiourea moiety has a stronger anion-binding capability than the urea.[72] This difference has been explained in terms of the higher acidity of thioureas as compared to ureas.[73] Compared to the linker 5-amino-2,4-dinitro-aniline discussed in the previous chapter, the urea was expected to result in macrocycles **43** with a shorter distance between the two naphthalene units as the urea is a slightly shorter linker than **30** (Scheme 4-1).



Scheme 4-1: General synthesis of bis-urea macrocycles.

Conformational analysis was performed with a simplified bis-urea macrocycle based on building block **23e** where the benzyl ethers of the amino acid side chains were replaced with methyl ethers. Monte Carlo simulations using the program MacroModel, based on the force field MMFF94s with chloroform as solvent, yielded 51 different conformers with an energy within 5 kcal/mol relative to lowest energy

conformer **C4** (Figure 4-1). These conformers were all small variations of the cleft-like **C4** and differed mostly in the orientation of the urea linkage as well as the amino acid side chains.

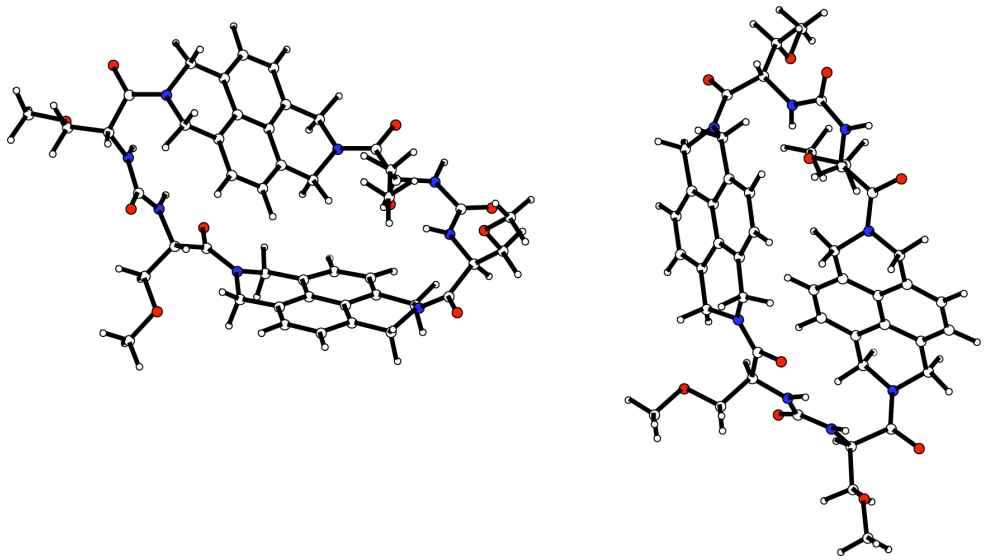


Figure 4-1: Lowest energy conformer **C4** of a bis-urea macrocycle as seen from two different angles.

The distance between the two aromatic diamines in conformer **C4** was measured at three positions (see Figure 3-4) as mentioned in chapter 3. The distances between the two pairs of benzylic nitrogens (d_1 and d_2) were found to be 6.7 Å and 7.4 Å, respectively, and the distance between the centroid positions of the aromatic moieties (d_3) was measured to 7.0 Å. These measurements indicate that the aromatic diamines actually are more distant to each other in bis-urea macrocyles **43** than in nitroanilines **37** despite the use of a shorter urea linker. This contradiction may be explained with conformational rigidity, the bis-ureas **43** have a more strained and rigid macrocyclic ring structure where the nitroanilines **37** have a larger, more flexible ring structure which allows for a certain amount of conformational collapse.

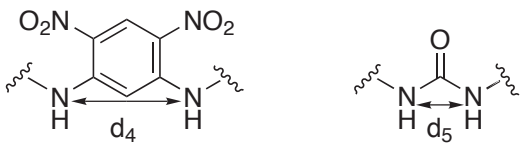
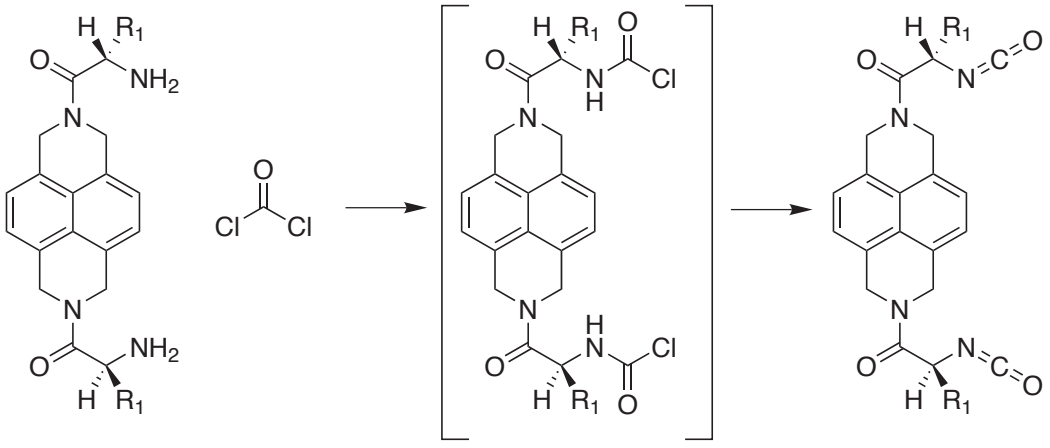


Figure 4-2: Measurement of nitrogen distance in linkers.

Within this context, it should be noted that the distance (Figure 4-2) between the two aniline nitrogens (d_4) of macrocycles **37** is 4.9 Å and the corresponding nitrogen distance (d_5) of bis-ureas **43** is 2.3 Å. These distances are not absolute values as the nitrogen distance of different conformers may deviate 0.1 Å.

Introduction to urea cross-linkers

Several different analogues of the cross-coupling reagent **41** have been employed in organic synthesis, one of the simplest examples is phosgene **44** which shows a strong reactivity towards nucleophiles (Scheme 4-2). The major drawback of phosgene, apart from its toxicity and volatility, is poor selectivity towards monosubstitution. The carbamoyl chloride **45** decomposes more or less spontaneously into isocyanate **46**, which also has a strong reactivity towards nucleophiles. Although methods have been published on the synthesis and use of peptide isocyanates with **44**,[74] this reagent was never employed as a cross-linking agent in this project as the synthesis of **46** most likely would be hampered by side-reactions such as polymerisation.



Scheme 4-2: Conversion into bis-isocyanates using phosgene.

Some other approaches to the bis-isocyanate **46** or its isothiocyanate analogue were evaluated. The reagent diiodosilane[75] reportedly provides an attractive route to isocyanates directly from Boc-protected amino acids, though attempts to produce **46**

from compound **17c** with diiodosilane failed. Attempts to produce bis-isothiocyanate from **23a** and carbon disulfide were also unsuccessful.[72]

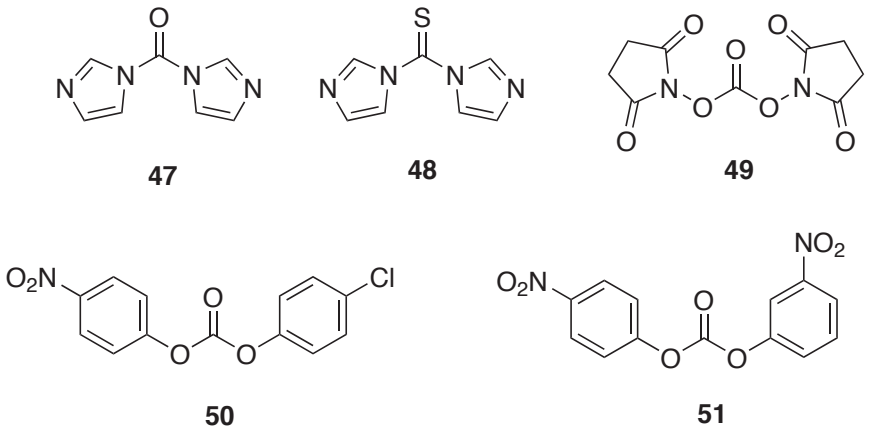
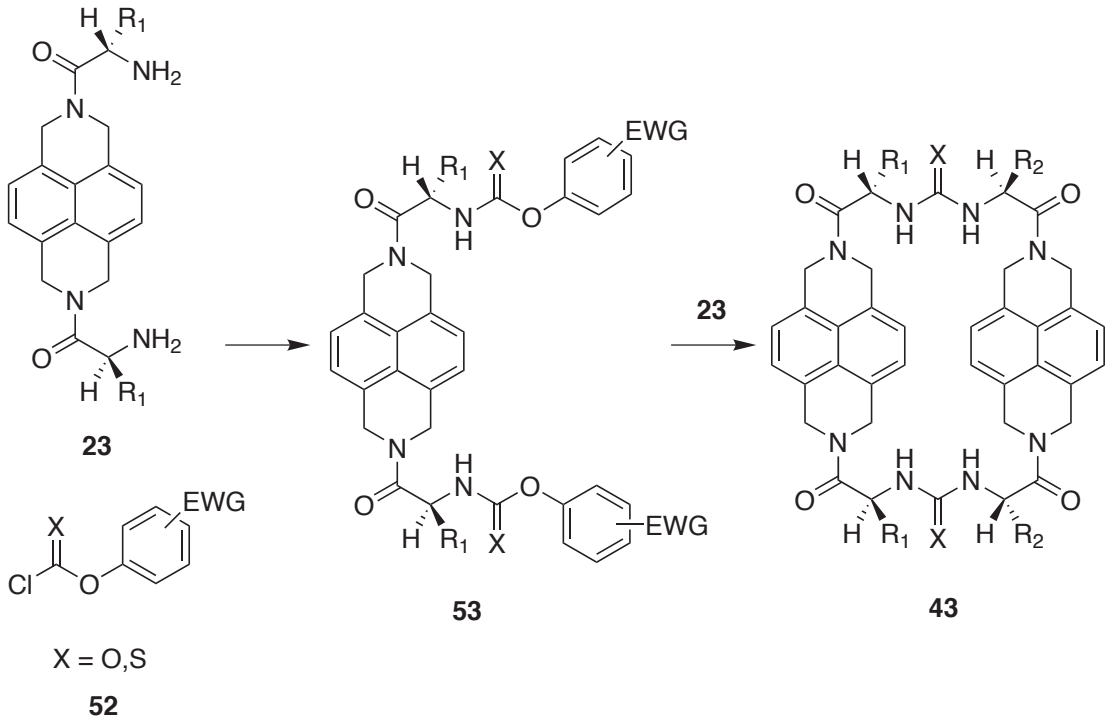


Figure 4-3: Crosslinkers evaluated and discarded.

Several different alternatives to **44** in the synthesis of bis-(thio)ureas have been reported[76] and a few of these were evaluated with slightly disappointing results (Figure 4-3). Carbonyldiimidazole **47** and its sulphur analogue **48** yielded crude materials which showed no signs of intermediate **42**, succinimidyl carbonate **49** showed poor reactivity towards dinucleophiles **23**, perhaps due to the poor solubility of **49**. Mixed aryl carbonates **50** and **51**[77] were easily prepared from 4-chlorophenol or 3-nitrophenol and 4-nitrophenyl chloroformate, though the resulting carbonates **50** and **51** showed poor reactivity towards dinucleophiles **23**.

Aryl chloroformate as cross-linking agent

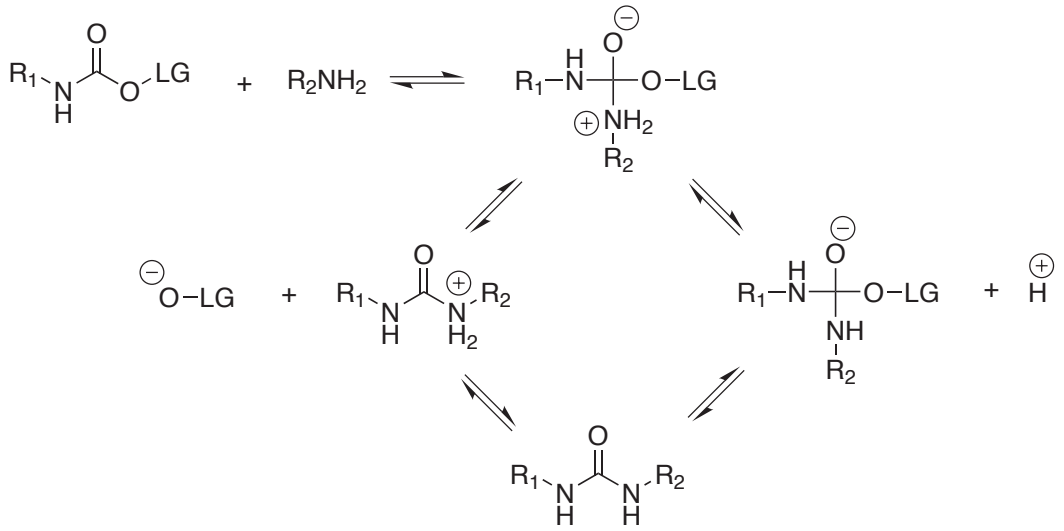
Somewhat better results were obtained in the reaction with **23** and aryl chloroformates **52** (Scheme 4-3). Although repeated attempts of reacting **23** with 4-nitrophenyl chloroformate failed to produce any 4-nitrophenyl carbamate **53**, small amounts of macrocycle **43** were detected in the reaction mixtures by MALDI-TOF, thus indicating 4-nitrophenyl carbamate **53** to be overly reactive towards dinucleophile **23**. On the other hand, phenyl chloroformate reacted smoothly at room temperature with **23** to produce good yields of unsubstituted phenyl carbamates **53**, these carbamates did however require extended refluxing conditions in THF to produce macrocycles **43**.



Scheme 4-3: Synthesis of bis-ureas and bis-thioureas with aryl chloro(thiono)formates.

It should be noted that the reactivity of phenyl carbamates versus amine nucleophiles is dependent on the solvent, the synthesis of ureas from unsubsituted phenyl carbamates is reported to proceed much faster in DMSO than THF.[78] The solvent DMSO is however somewhat impractical for the diluted conditions necessary in macrocyclisations.

The reaction between an amine and a chloroformate is generally considered to proceed through a stepwise addition-elimination mechanism including a tetrahedral intermediate,[79] but the mechanism of the aminolysis of carbamates into ureas has been subject to some discussion. Carbamate aminolysis has been suggested[80] to proceed via an isocyanate intermediate obtained through E2- or E1cB-elimination. Later investigations[81] have however shown the aminolysis to proceed through an stepwise addition-elimination mechanism where breakdown of the zwitterionic tetrahedral intermediate is the rate-limiting step (Scheme 4-4).

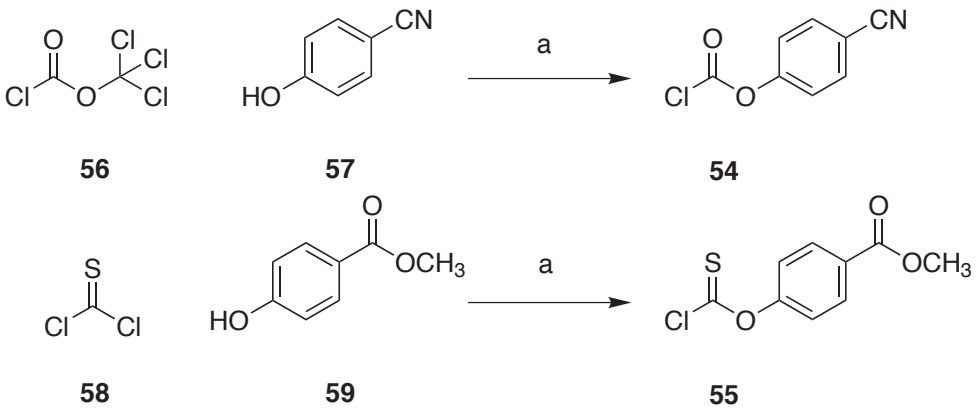


Scheme 4-4: Mechanism established for the aminolysis of carbamates.

While the elimination of the tetrahedral intermediate is subject to base catalysis, it is also dependent on the nature of the leaving group. Based on the poor reactivity of phenyl carbamate **53** and the excessive reactivity of corresponding 4-nitrophenyl carbamate, a closer investigation of differently substituted phenyl carbamates **53** was initiated with electron-withdrawing groups (EWGs) of intermediate power. The purpose was to find an aryl carbamate with optimal balance between stability and reactivity, *i.e.* a carbamate **53** stable enough to isolate from the reaction between dinucleophile **23** and aryl chloroformate **52**, yet reactive enough to produce reasonable yields of macrocycles **43** under mild reaction conditions.

Synthesis of active carbamates

All of the aryl chloro(thiono)chloroformates necessary were commercially available with the exception of 4-cyanophenyl chloroformate **54** and 4-methoxycarbonylphenyl chlorothionoformate **55** (Scheme 4-5). The chloroformate **54** was easily prepared[82] from diphosgene **56** and 4-cyanophenol **57**, chlorothionoformate **55** was obtained from thiophosgene **58** and methyl 4-hydroxybenzoate **59** under similar conditions. Both **54** and **55** were contaminated with small amounts of diisopropylethylammonium hydrochloride salt, but this was assumed to be of less concern during the synthesis of corresponding carbamates.

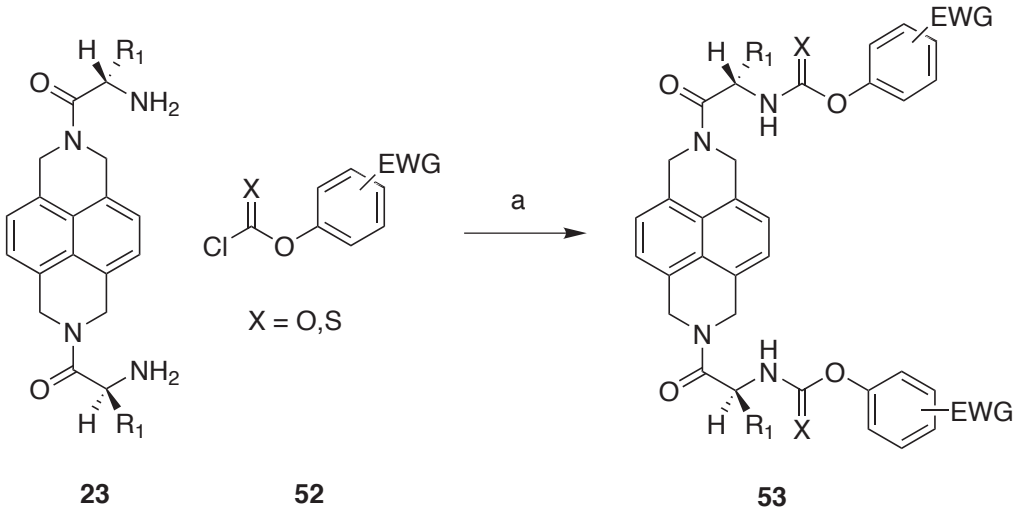


56 **57** **54**

58 **59** **55**

Scheme 4-5: Synthesis of cross-linkers. a: *i*-Pr_2EtN, CH_2Cl_2

Reactions between diamines **23•TFA** and the different chloro(thiono)formates were performed at room temperature in THF in the presence of caesium carbonate and were generally finished within a reaction time of 2 hours (Scheme 4-6). The reactions were easily monitored by both TLC and MALDI-TOF as well as visual appearance, almost all of the (thiono)carbamates **53** precipitated from the reaction mixture.



23 **52** **53**

Scheme 4-6: Synthesis of active carbamates **53**. a: Cs_2CO_3, THF, rt.

Most of the (thiono)carbamates **53b-r** were fairly easy to isolate by flash chromatography, though **53i**, as well as **53k** and **53o**, decomposed significantly during workup of the reactions (Table 4-1). The carbamates **53** were somewhat difficult to characterise by spectroscopy due to their inherent reactivity. Most of the carbamates were detected with MALDI-TOF, though ^{1}H-NMR-analyses often indicated a few percents degradation of **53** visible as a contamination with EWG-

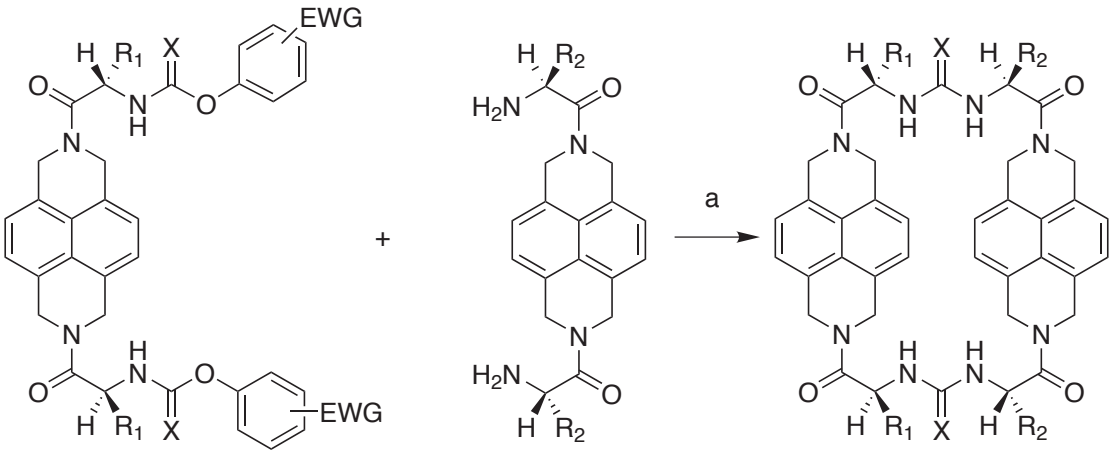
substituted phenol. Rather than performing more elaborate analyses, such as HR-MS, the carbamates were used immediately in the next step.

Table 4-1: Active carbamates prepared.

carbamate	diamine	R_1	X	EWG	yield (%)
53a	**23e**	CH_2OBn	O	4-NO_2	0
53b	**23e**	CH_2OBn	O	4-H	71
53c	**23c**	$(CH_2)_2CO_2Bn$	O	4-H	84
53d	**23e**	CH_2OBn	O	4-F	73
53e	**23e**	CH_2OBn	O	4-Cl	79
53f	**23c**	$(CH_2)_2CO_2Bn$	O	4-Cl	89
53g	**23d**	CH_2CO_2Bn	O	4-Cl	89
53h	**23c**	$(CH_2)_2CO_2Bn$	O	4-CO_2CH_3	72
53i	**23c**	$(CH_2)_2CO_2Bn$	O	4-CN	59
53j	**23c**	$(CH_2)_2CO_2Bn$	S	4-Cl	84
53k	**23c**	$(CH_2)_2CO_2Bn$	S	2,3,4,5,6-F	54
53l	**23c**	$(CH_2)_2CO_2Bn$	O	4-F	79
53m	**23c**	$(CH_2)_2CO_2Bn$	S	4-CO_2CH_3	41
53n	**23d**	CH_2CO_2Bn	O	4-CO_2CH_3	68
53o	**23d**	CH_2CO_2Bn	S	2,3,4,5,6-F	64
53p	**23g**	$(CH_2)_4NHZ$	O	4-CO_2CH_3	75
53q	**23h**	$(CH_2)_2CO_2tBu$	O	4-CO_2CH_3	52
53r	**23c**	$(CH_2)_2CO_2Bn$	O	3-CF_3	61

Synthesis of ureas - evaluation of active carbamates

Reactions between active (thiono)carbamates **53** and dinucleophiles **23** (**23•TFA** unless otherwise noted) were performed with diluted conditions at ambient temperature, the concentrations of **53** and **23** were in the range of 0.5-1.0 mM (Scheme 4-7). The reactions were conveniently followed with MALDI-TOF where the caesium carbonate employed as base in the reactions resulted in beneficial caesium-peaks. During MALDI-TOF-analyses it was sometimes almost impossible to detect the protonated molecular ion $[M+H]^+$ of a compound with molecular weight M, the compound was perhaps only detected as a sodium ion $[M+Na]^+$. The presence of caesium carbonate did however give rise to caesium ions $[M+Cs]^+$ which together with $[M+Na]^+$ confirmed the presence of molecule M despite the absence of $[M+H]^+$.



53 **23** **43**

Scheme 4-7: Synthesis of macrocyclic bis(thio)ureas. a: Cs_2CO_3, THF, rt.

Table 4-2: Preparation of macrocyclic bis-ureas (X=O).

carb.	amine	**prod**	R_1	R_2	EWG	time (h)	yield(%)[a]
53a	**23e**	**43a**	CH_2OBn	CH_2OBn	4-NO_2	48	trace
53b	**23e**	**43a**	CH_2OBn	CH_2OBn	4-H	48[b]	43
53b	**23a**	**43b**	CH_2OBn	H	4-H	48	0
53b	**23c**	**43c**	CH_2OBn	$(CH_2)_2CO_2Bn$	4-H	50	0
53b	**23c**	**43c**	CH_2OBn	$(CH_2)_2CO_2Bn$	4-H	72	0
53d	**23e**	**43a**	CH_2OBn	CH_2OBn	4-F	96	0
53d	**23e**	**43a**	CH_2OBn	CH_2OBn	4-F	22	0
53d	**23e**	**43a**	CH_2OBn	CH_2OBn	4-F	20	0
53l	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-F	28	40
53f	**23c**	**43d**	$(CH_2)_2CO_2Bn$	$(CH_2)_2CO_2Bn$	4-Cl	21	36
53f	**23c**	**43d**	$(CH_2)_2CO_2Bn$	$(CH_2)_2CO_2Bn$	4-Cl	20	51[c]
53f	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-Cl	22	56
53f	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-Cl	48	0
53g	**23e**	**43e**	CH_2CO_2Bn	CH_2OBn	4-Cl	24	55
53i	**23c**	**43d**	$(CH_2)_2CO_2Bn$	$(CH_2)_2CO_2Bn$	4-CN	22	28
53h	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	6	25
53h	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	19	66
53h	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	7	52[c]
53h	**23d**[d]	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	24	0
53h	**23d**[d]	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	5	20
53h	**23d**[d]	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	6	0
53h	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	7.5	27
53h	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	5	36
53h	**23a**	**43j**	$(CH_2)_2CO_2Bn$	H	4-CO_2CH_3	23	33[c]
53n	**23d**	**43k**	CH_2CO_2Bn	CH_2CO_2Bn	4-CO_2CH_3	6	61
53p	**23f**	**43m**	$(CH_2)_4NHZ$	$(CH_2)_3NHC(NZ)NHZ$	4-CO_2CH_3	60	0[e]
53q	**23h**	**43n**	$(CH_2)_2CO_2tBu$	$(CH_2)_2CO_2tBu$	4-CO_2CH_3	22	23
53q	**23h**	**43n**	$(CH_2)_2CO_2tBu$	$(CH_2)_2CO_2tBu$	4-CO_2CH_3	21	27
53r	**23d**	**43f**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	3-CF_3	6	30

a: Yield of material after flash chromatography. *b*: Refluxing conditions. *c*: Confirmed by HRMS.

d: Using **23•HCl**. *e*: Failure in deprotection to **23f•TFA**.

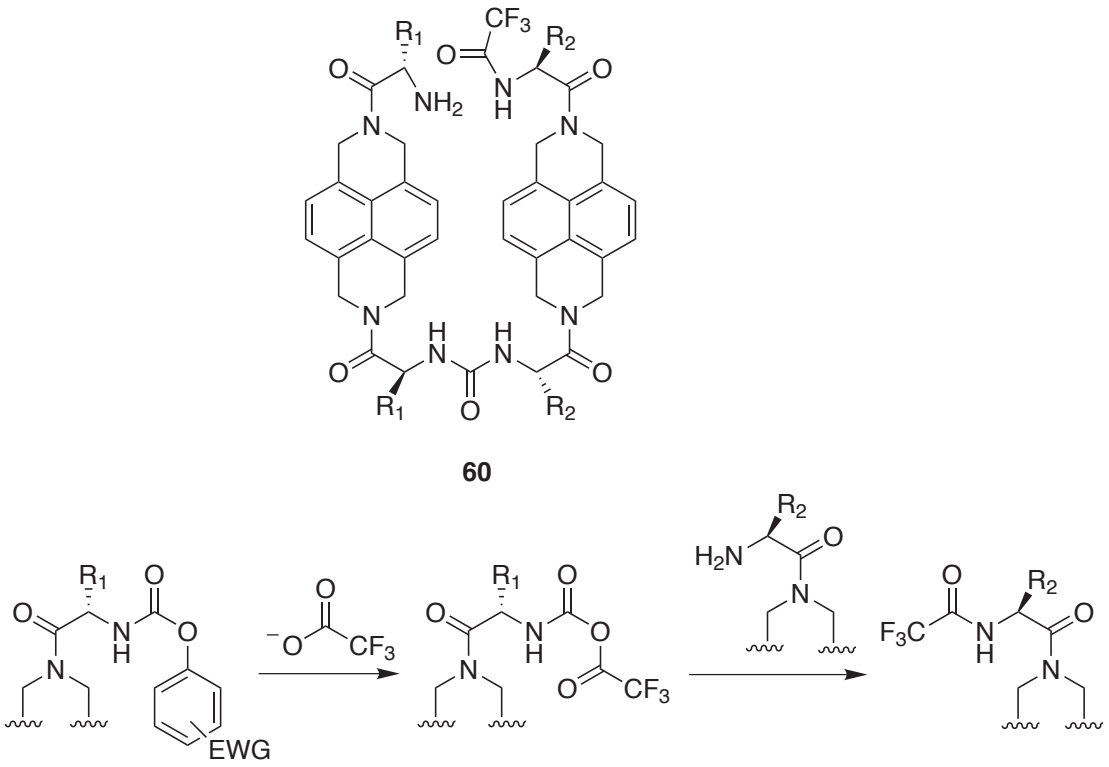
The phenyl carbamates which showed the best balance between stability and reactivity were **53h,n,q**,**r**, *i.e.* 4-methoxycarbonyl- or 3-trifluoromethyl-substituted phenyl carbamates (Table 4-2). These carbamates were fairly easy to prepare and gave good yields of macrocyclic bis-ureas after a few hours reaction at room temperature. The 4-cyano-substituted carbamate **53i** gave similar yields of macrocyclic compounds though the utilisation of a non-commercially available chloroformate, as well as stability issues, made this carbamate less appealing. The 4-halogen-substituted carbamates **53d,f,g,l** were easily prepared and gave good yields of macrocyclic products although at a somewhat slower reaction rate than the more reactive carbamates **53h,n,q,r**.

Table 4-3: Preparation of macrocyclic bis-thioureas (X = S).

carb.	amine	**prod**	R_1	R_2	EWG	time (h)	yield(%)[a]
53m	**23d**	**43g**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-CO_2CH_3	6	54[b]
53k	**23d**	**43g**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	2,3,4,5,6-F	24	30
53o	**23e**	**43l**	CH_2CO_2Bn	CH_2OBn	2,3,4,5,6-F	6	22[b]
53j	**23d**	**43g**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-Cl	22	39
53j	**23c**	**43h**	$(CH_2)_2CO_2Bn$	$(CH_2)_2CO_2Bn$	4-Cl	23	59[b]
53j	**23e**	**43i**	$(CH_2)_2CO_2Bn$	CH_2OBn	4-Cl	19	52[b]
53j	**23d**	**43g**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-Cl	20	33
53j	**23d**[c]	**43g**	$(CH_2)_2CO_2Bn$	CH_2CO_2Bn	4-Cl	24	0

a: Yield of material after flash chromatography. *b*: Confirmed by HRMS. *c*: Using **23•HCl**

Not surprisingly, the synthesis of macrocyclic bis-thioureas via thionocarbamates showed a similar pattern of reactivity (Table 4-3). The 4-methoxycarbonyl-substituted thionocarbamate **53m** gave a good yield of macrocycle **43g** after a few hours of reaction with diamine **23d**. To avoid the hazardous synthesis of **55** involving the repulsive (and highly toxic) reagent **58,** some thionocarbamates prepared with commercially available chlorothionoformates were investigated. As the pentafluorosubstituted thionocarbamates **53k,o** decomposed significantly, the synthesis of macrocyclic bis-thioureas was preferentially performed with 4-chloro-substituted thionocarbamates such as **53j**.



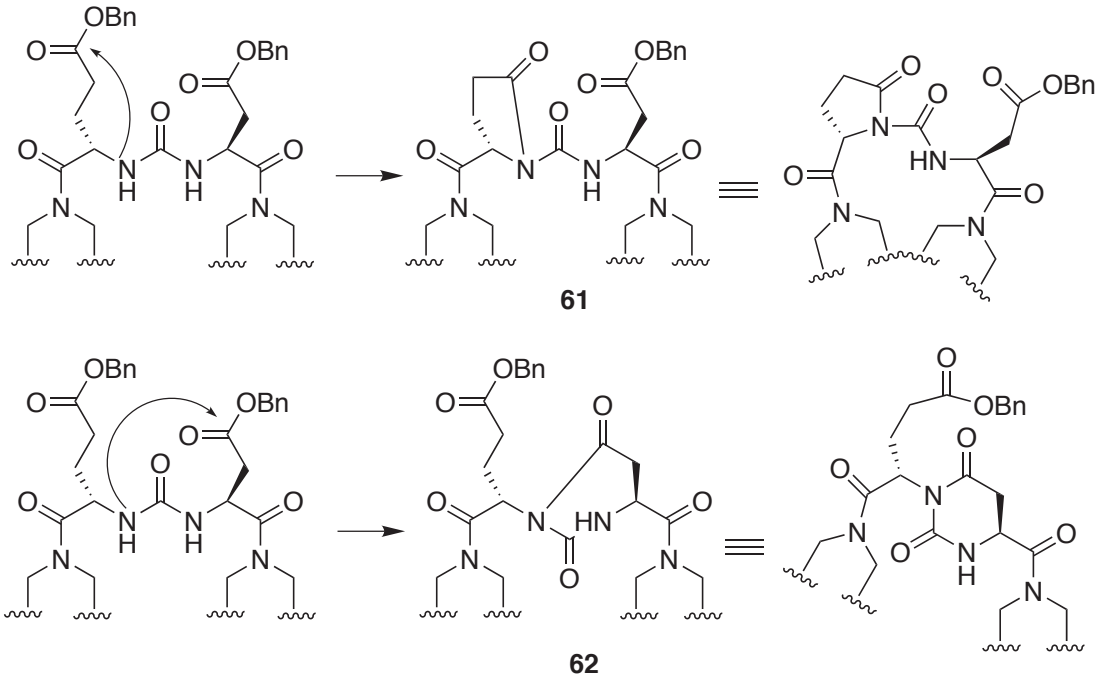
60

Scheme 4-8: Proposed structure and formation of byproduct observed during macrocyclisations.

While following the macrocyclisations with MALDI-TOF, a byproduct with molecular weight 70 Daltons higher than the desired product was observed (Scheme 4-8). This byproduct was difficult to separate from the product and was never characterised with NMR-spectroscopy. Compound **60** is a likely structure of the byproduct, as it has a calculated molecular weight of 70 Daltons higher than the desired product and its appearance may be derived from residual trifluoroacetate in dinucleophile **23**. Byproduct **60** is presumably formed by nucleophilic attack of trifluoroacetate in the active carbamate which gives a mixed anhydride between carbamic acid and trifluoroacetic acid. This mixed anhydride serves as a trifluoroacetylating agent towards an amino nucleophile thus giving **60**. Other examples of trifluoroacetylated byproducts in peptide synthesis have been reported.[83,84]

The formation of byproduct **60** could be suppressed, but not completely eliminated, by scrupulous evaporation of the diamino salts **23•TFA** to remove excessive TFA. By performing deprotection of Boc-protected building blocks **17** with HCl•Et_2O instead of TFA the byproduct was completely eliminated. The diamino salts **23•HCl** were on the other hand poorly soluble in THF which caused the macrocyclisations to proceed

slower. Altering the solvent to DMF gave better solubility of diamino hydrochloride salts and thus faster formation of macrocyclic compounds. Unfortunately, another complicating byproduct was observed by MALDI-TOF in macrocyclisations performed in DMF with diamino salts **23•HCl** (Scheme 4-9). This byproduct, with a possible structure of **61** or **62**, had a molecular weight of 108 Daltons lower than the desired product which indicates loss of a benzyl alcohol from the side-chain of glutamic **61** or aspartic acid **62** by intramolecular cyclisation similar to the aspartimide often encountered in peptide synthesis.[85,86] Whether byproducts **61,62** are formed from a macrocyclic compound or a linear intermediate is uncertain, though by substituting the benzyl groups with more sterically hindered protective groups such as cyclohexyl[87] or 2,4-dimethyl-3-pentyl[88] the formation of **61-62** should be largely diminished.



Scheme 4-9: Another possible byproduct formed.

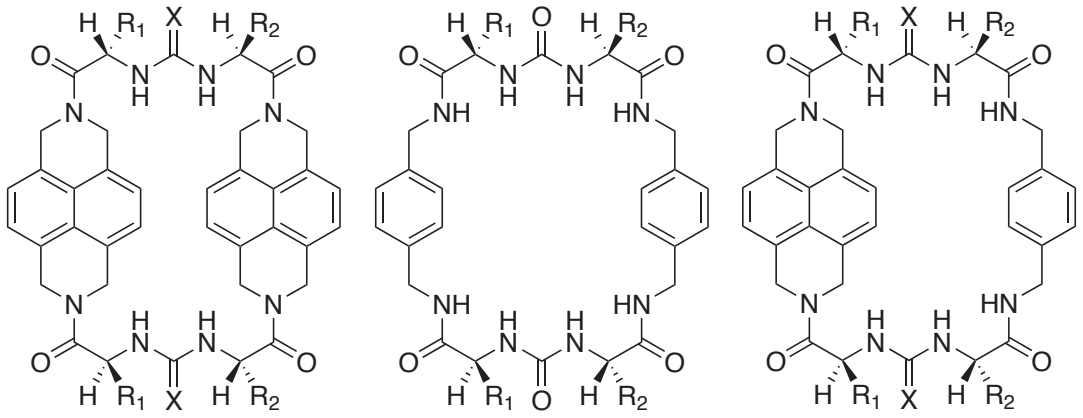
Similar to the macrocyclic compounds **37** discussed in the previous chapter, macrocycles **43** proved difficult to analyse with ^{1}H-NMR due to poor resolution. To make matters worse, the compounds **43** have neither yielded satisfactorly analyses with ^{13}C-NMR nor satisfactorly chromatography results with reversed-phase HPLC. Mass spectrometry analyses have however indicated the presence of macrocycles **43**. Apart from the MALDI-TOF analyses of reaction mixtures and products, ES-HRMS

have confirmed the composition of compounds **43d,f,g,h,i,j,l**. The macrocyclic bis-(thio)ureas **43** also seem to be less stable upon handling and storage than macrocycles **37**, possibly due to larger ring strain in macrocycles **37** caused by the shorter crosslinking reagent.

Protective group chemistry

Removal of the protective groups from macrocycles **43** showed to be equally difficult as observed with macrocycles **37**. Palladium catalysed debenzylation with hydrogen gas or ammonium formate under neutral conditions at room temperature proceeded very slowly and the small amounts of deprotected macrocycle formed were accompanied by fragmentation of the desired macrocycle into smaller molecules as indicated by MALDI-TOF-analyses. Palladium catalysed transfer hydrogenation under acidic conditions with formic acid as hydrogen donor proceeded much faster, though formation of byproducts did occur to a large extent. Attempts to debenzylate with TMS-I or alkaline ester hydrolysis with LiOH or KOH were equally unsuccessful as fragmentation to smaller molecules proceeded in parallel to deprotection.

To overcome the problems associated with deprotection of the benzyl groups, as well as byproducts formed during macrocyclisations, bis-urea **37n** was prepared with side-chains protected as *t*-butyl esters. This protective group is normally removed by mild acidolysis and is less sensitive to nucleophilic attack than the benzyl ester due to steric hindrance. Although the synthesis of **37n** generated less byproducts, as indicated by MALDI-TOF, attempts to remove the *t*-butyl esters with diluted TFA were unfortunately unsuccessful as the bis-urea **37n** decomposed into smaller fragments under the acidic conditions.



43 **63** **64**

Figure 4-4: Bis-(thio)ureas prepared (**43** and **63**) and targeted (**64**).

Needless to say, the bis-(thio)ureas **43** are more difficult to prepare and handle than the nitroanilines **37**, presumably due to stronger ring strain in **43** caused by the smaller (thio)urea linker. Other macrocyclic bis-ureas **63** have been prepared in our laboratory (Figure 4-4),[24] these bis-ureas are based on a less rigid diamine and are easier to prepare and handle than **43**, presumably due to larger conformational flexibility. The larger flexibility of **63** does however come at the price of not having a conformationally defined binding pocket. The mixed bis-(thio)urea **64** might very well show to be a good compromise between the rigid but unstable **43** and the stable but flexible **63**.

5: Mixed diamine macrocycles

The overall structure of the macrocycle **8** contains five obvious positions of diversification (Figure 5-1), namely the two diamines (arrow a,e), the two amino acids (arrow b,d) and the cross-linker (arrow c). Macrocycles **37** and **43** discussed in chapters 3 and 4 are examples of diversification with respect to cross-linker and amino acids. Mixed diamine macrocycles are accessible by substituting one of the diamines with a diamine different from **9**.

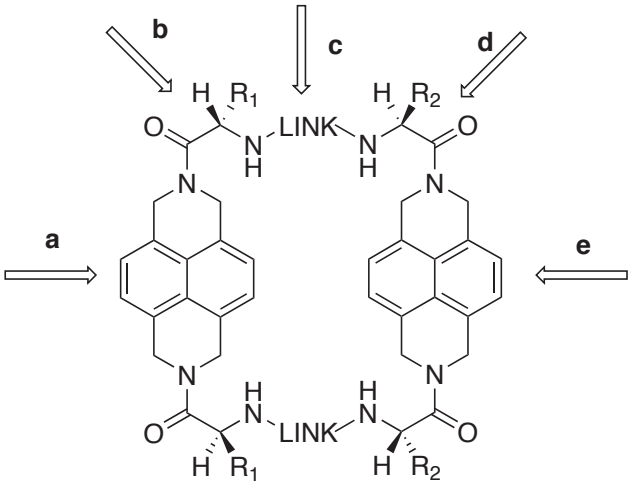
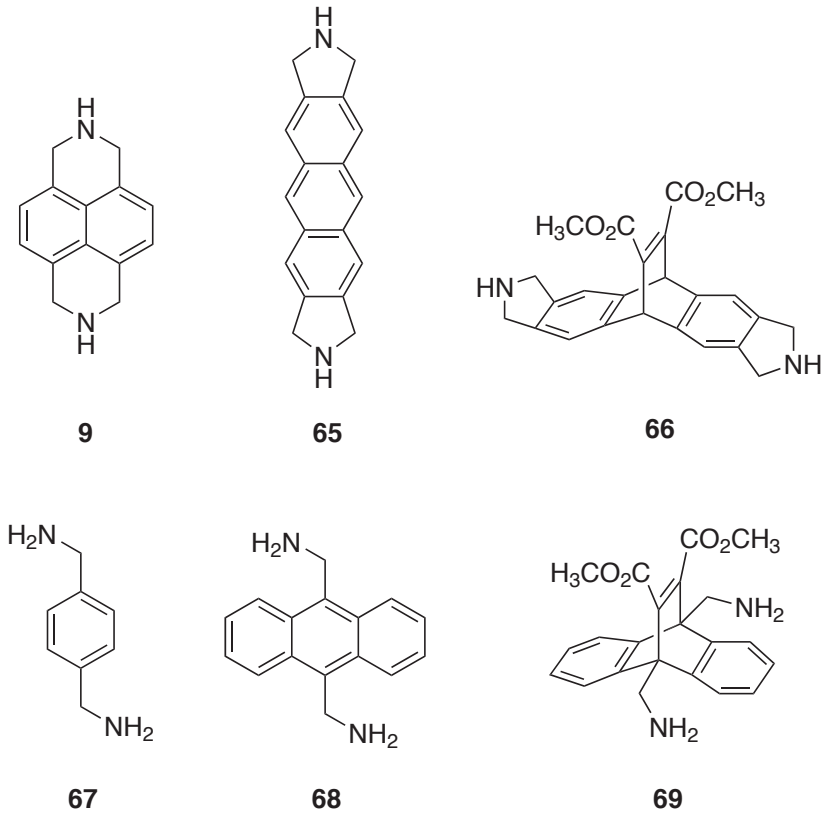


Figure 5-1: Positions of diversifications in **8**.

Different diamines

Apart from diamine **9**, other aromatic diamines have been prepared and acylated with amino acids in our laboratory, thus offering a variety of building blocks suitable for incorporation in macrocycles (Figure 5-2). Diamine **65**, as well as its Diels-Alder adduct **66**, are two alternative scaffolds (Paper I). Both **65** and **66** should provide rigid macrocycles similar to **9**, as all of these compounds are secondary diamines. More flexible macrocycles may be obtained with primary diamines. Besides the commercially available *p*-xylylenediamine **67**,[24] anthracene-based diamines **68** and **69**[89,90] may be incorporated into macrocycles. Although the diamine **69** spontaneously undergoes intramolecular ring closure to form a bis-lactam under basic conditions, the building block **69** may be incorporated in macrocycles by preparing macrocycles with

diamine **68** and performing a Diels-Alder reaction with these macrocycles and dimethyl acetylenedicarboxylate.



9 **65** **66**

67 **68** **69**

Figure 5-2: Diamines of potential use in macrocyclisations.

These six different diamines may be acylated with the 20 standard amino acids[2] and conjugated with the three linkers dinitrodianiline, urea and thiourea to give a total number of 43 200 different macrocycles. While the synthesis of all those compounds is beyond the scope of this thesis, some attempts were performed at producing mixed macrocycles incorporating two different diamines.

The synthesis of these mixed macrocycles was focused on using 1,5-difluoro-2,4-dinitrobenzene (**30**) as cross-linking agent, as the synthesis of bis-dinitrodianilines **37** had been slightly more reliable than the bis-(thio)ureas **43**. Serine, with its sidechain protected as a benzyl ether, was chosen as amino acid in the first mixed macrocycles as this amino acid repeatedly has yielded products with good solubility and its ether protected sidechain is unlikely to obstruct the cyclizations.

Flexible mixed macrocycle

As mentioned in the previous chapters, macrocycles incorporating two units of diamine **9** were difficult to handle with respect to stability, possibly due to ring strain. By introducing the more flexible diamine **67** into the macrocycle, the resulting mixed macrocycle could be expected to be less prone to decomposition during deprotection of amino acid side chains.

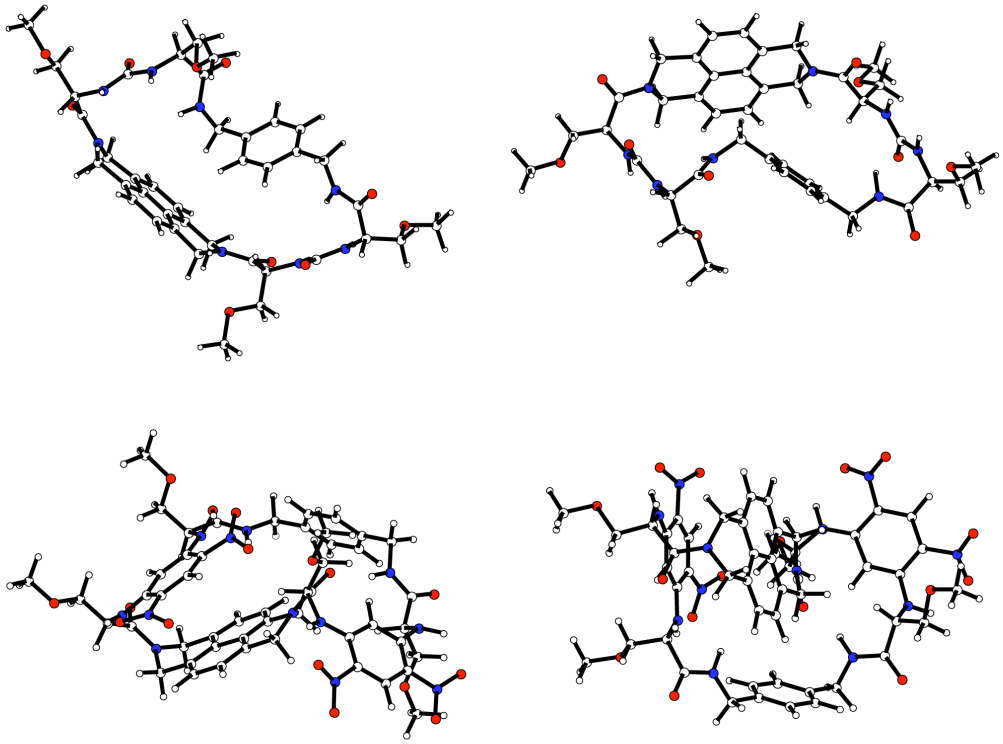


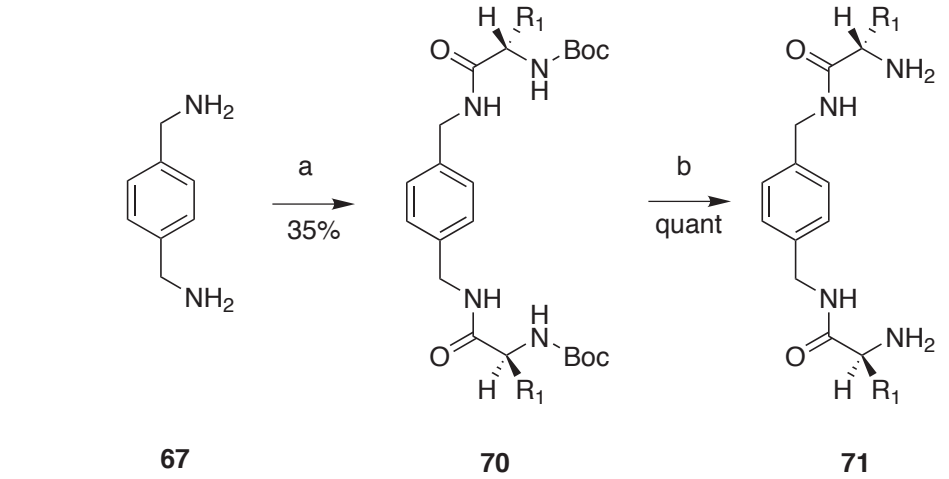
Figure 5-3: Top left, right: lowest energy conformer **C5** of mixed bis-urea. Bottom left, right: lowest energy conformer **C6** of mixed bis-nitroaniline.

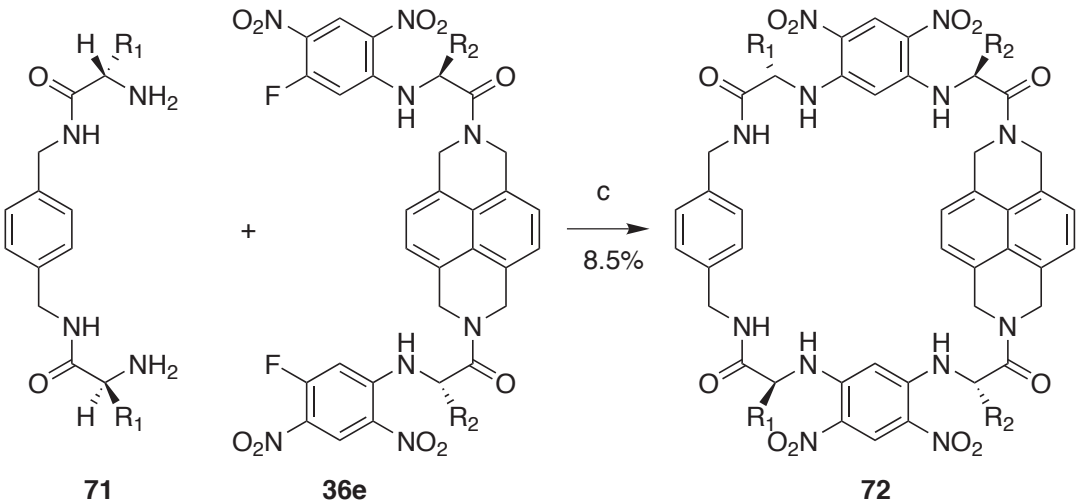
Conformational analyses were performed on both a mixed bis-urea and a mixed bis-nitroaniline incorporating diamines **9** and **67**, as well as serine residues. Monte Carlo simulations using the program MacroModel, based on the force field MMFF94s with chloroform as solvent, yielded for the bis-urea 615 different conformers with an energy within 5 kcal/mol relative to the lowest energy conformer **C5** as seen from two different angles in Figure 5-3. As expected, this mixed cyclophane had a larger conformational flexibility than the bis-urea **43** and gave a more diverse range of conformers with large variations in the orientation of the two diamines. In the case of

the mixed bis-nitroaniline, similar calculations yielded 276 different conformers with an energy within 5 kcal/mol relative to the lowest energy conformer **C6**. These conformers were equally diverse, though most of them had at least two aromatic rings stacked on top of each other. The distances between the aromatic diamines were measured as described in chapter 3 (Table 5-1).

Table 5-1: Distances measured between aromatic diamines.

Conformer	d_1 (Å)	d_2 (Å)	d_3 (Å)
C5	6.4	6.0	4.8
C6	4.6	6.3	6.6





Scheme 5-1: Synthesis of mixed macrocycle **72**. $R_1 = R_2 = CH_2OBn$. a: Boc-Ser(OBn)-OH, DIC, HOBt, DMAP, THF, rt. b: TFA, Et_3SiH, CH_2Cl_2, rt. c: Cs_2CO_3, THF, reflux.

Acylation of diamine **67** with serine gave diacylated compound **70** in a modest yield of 35% which is far from optimised (Scheme 5-1). This particular reaction was performed with a slightly dated sample of the hygroscopic diamine **67** and the product **70** was somewhat difficult to separate from the diisopropylurea formed from DIC.

Deprotection of **70** with TFA in the presence of Et_3SiH proceeded smoothly and the resulting **71** was then reacted with **36e** under the usual conditions. The resulting yield of cyclophane **72** was somewhat lower than the yields obtained during syntheses of **37**, this unoptimised yield is likely to be improved in future attempts to prepare **72**. The composition of **72** has been confirmed with ES-HRMS, though the purity of this compound is yet to be determined with ^{13}C-NMR.

Rigid mixed macrocycle

Although the major reason for preparing mixed diamine macrocycles was to find macrocycles with a better stability than bis-dinitrodianilines **37** and bis-(thio)ureas **43**, this approach could also be used to enhance the rigidity of the macrocycles and result in potential hosts with a better definded binding cavity. The question thus arose as to whether the procedures for producing macrocycles established in chapter 3 and 4 are appropriate for producing even more rigid structures, structures such as a macrocycle incorporating diamines **9** and **65**.

Conformational analyses were performed with both a hybrid bis-urea and a hybrid bis-nitroaniline incorporating diamine **9** and **65** and serine residues. Once again, Monte Carlo simulations were performed with the program MacroModel, based on the force field MMFF94s with chloroform as solvent. The mixed bis-urea yielded 215 different conformers with an energy within 5 kcal/mol relative to the lowest energy conformer **C7** as seen from two different angles in Figure 5-4. These conformers showed only small variations in the orientation of the ureas and the amino acid side chains which indicate a most rigid structure, another indication of the rigidity is that the anthracene is slightly bent.

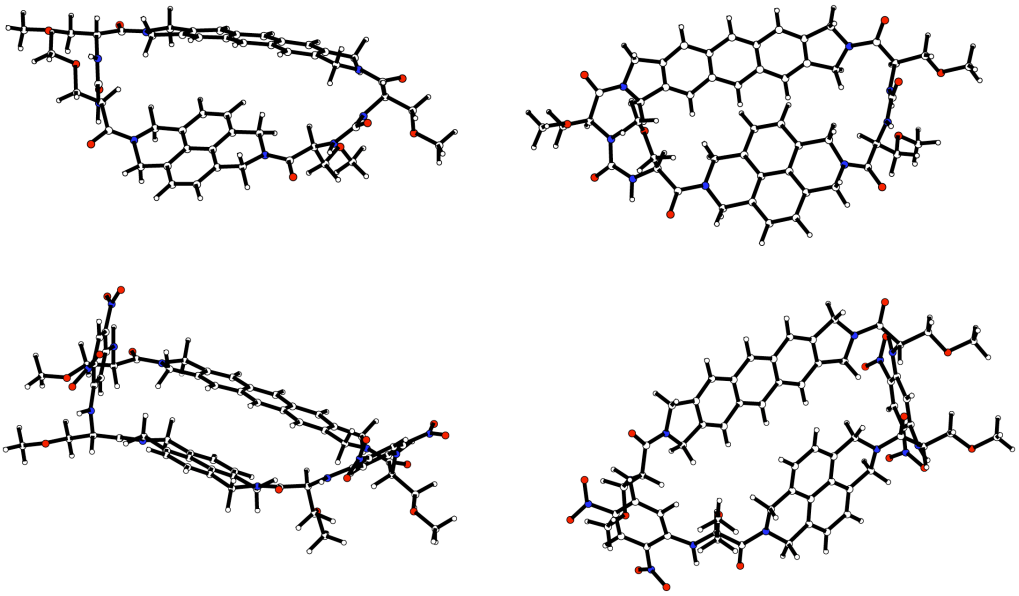


Figure 5-4: Top left, right: lowest energy conformer **C7** of a hybrid bis-urea. Bottom left, right: lowest energy conformer **C8** of a hybrid bis-nitroaniline.

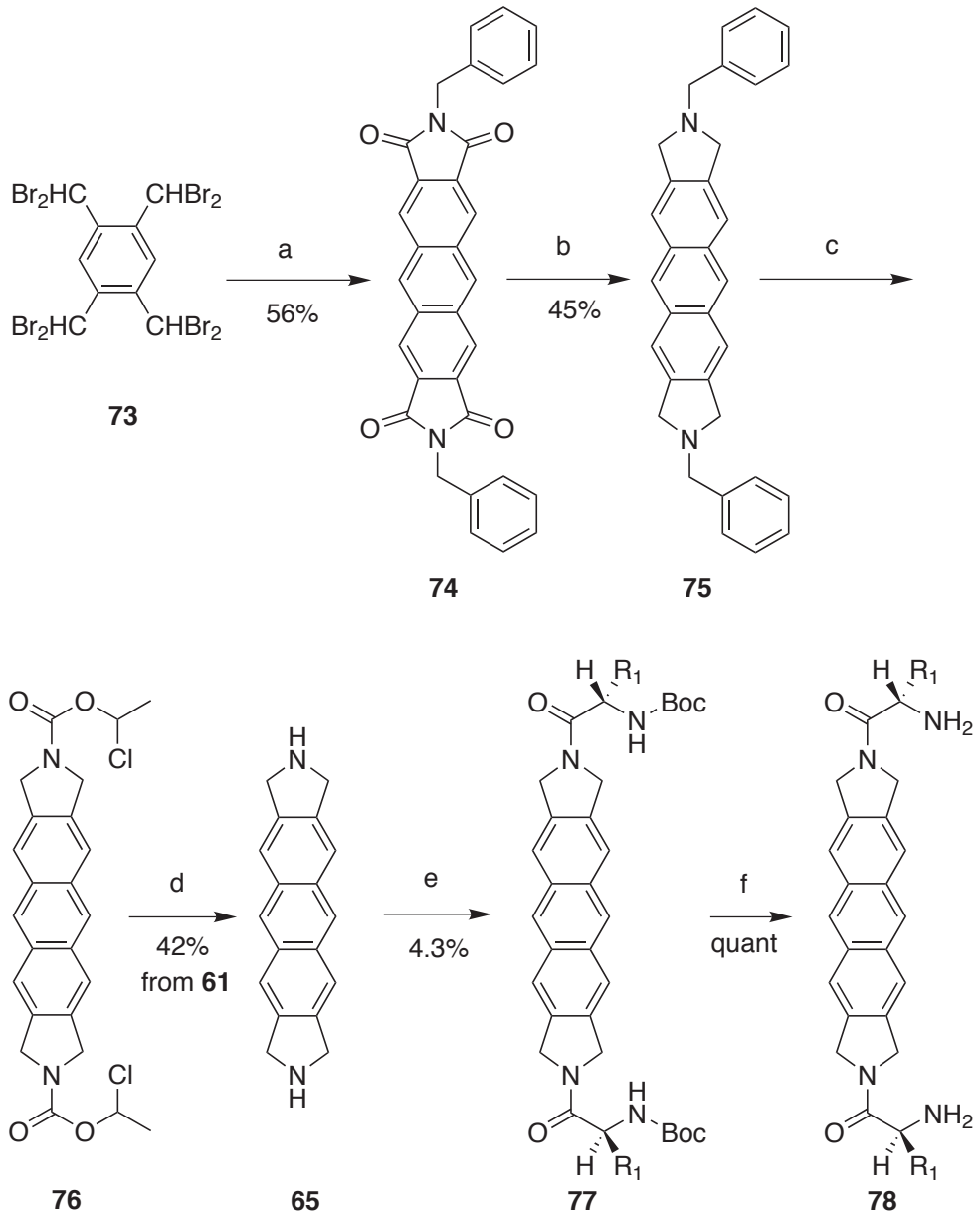
The bis-nitroaniline yielded 236 different conformers with an energy within 5 kcal/mol relative to the lowest energy conformer **C8** (Figure 5-4). Similar to the mixed bis-ureas, these conformers were only small deviations of the conformer **C8**, although the curvature of the anthracene was less pronounced. The distances between the two aromatic diamines in **C7** and **C8** were measured and found to be quite similar (Table 5-2).

Table 5-2: Distances measured between aromatic diamines.

Conformer	d_1 (Å)	d_2 (Å)	d_3 (Å)
C7	6.4	6.7	6.6
C8	5.9	7.5	6.5

The synthesis of a mixed macrocycle with the diamines **9** and **65** showed, as expected, to be more laborious (Scheme 5-2). The synthesis of building block **78** is largely outlined in paper I, though some minor adjustments were later found appropriate. Diimide **74** was readily obtained by reacting octabromide **73** with 2 equivalents of *N*-benzylmaleimide, the crude product was however purified by treatment with hot water followed by filtration and finally precipitation from acetone as the diimide **74** was difficult to precipitate from dioxane. Reduction of **74** into dibenzylated diamine **75** proceeded as expected, though the extractive workup with

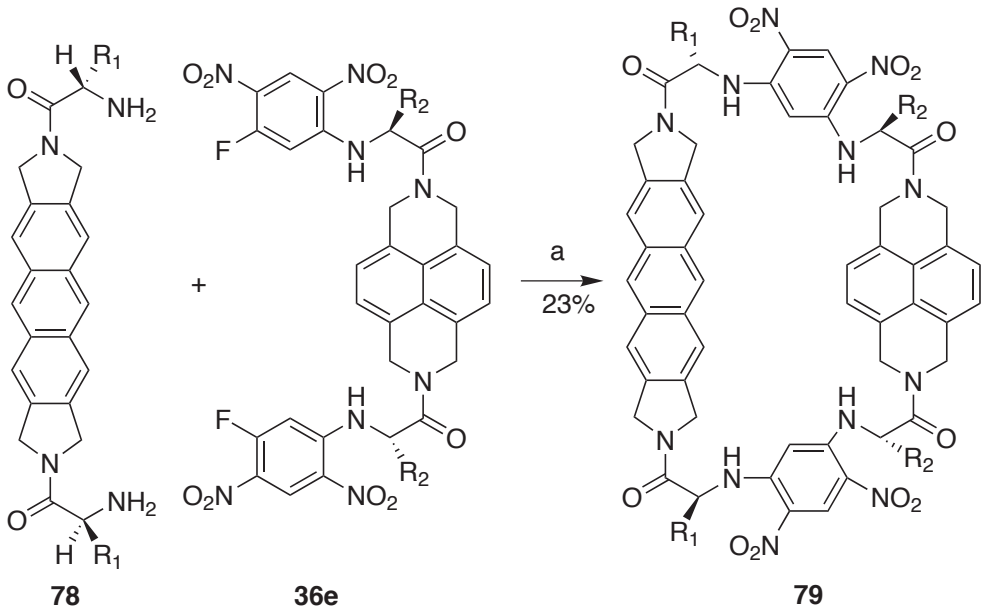
dichloromethane was difficult to reproduce, instead the crude material was purified by flash chromatography directly after quenching the reaction.



Scheme 5-2: Synthesis of building block **78**. $R_1 = CH_2OBn$. a: *N*-Benzylmaleimide, NaI, DMAc, 80°C. b: AlH_3, THF, reflux. c: 1-chloroethyl chloroformate, benzene, 1,2-dichloroethane, reflux. d: CH_3OH, reflux. e: Boc-Ser(OBn)-OH, DIC, HOBt, DMAP, Cs_2CO_3, CH_2Cl_2, THF, rt. f: TFA, Et_3SiH, CH_2Cl_2, rt.

Removal of benzyl groups with chloroethyl chloroformate was, as mentioned in chapter 2, an unreliable procedure. Although the yield obtained of diamine **65**, as its dihydrochloride salt, was similar to the yield originally reported, the purity of the **65** obtained to be used in macrocyclisations was lower than expected. Due to the limited amounts of starting material available no further optimisations were affordable, rather

the diamine **65** was used directly in the next step. Still, the acylative debenzylation could perhaps be more reliable with ethyl chloroformate and alkaline hydrolysis.



78 **36e** **79**

Scheme 5-3: Synthesis of rigid mixed macrocycle. $R_1 = R_2 = CH_2OBn$. a: Cs_2CO_3, THF, reflux.

Acylation of **65** gave compound **77** in an unimpressive yield of 4.3%, presumably due to low purity of **65**. Nevertheless, synthesis of **79** was attempted with **36e** and **78** under the usual conditions (Scheme 5-3) and resulted in a reasonable yield of the cyclophane **79**. The compostion of **79** has been confirmed with ES-HRMS, though the purity of this compound is yet to be determined with ^{13}C-NMR.

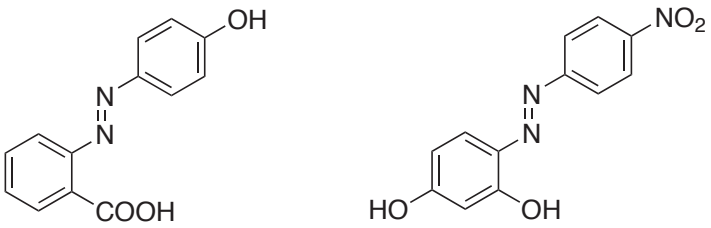
Conclusion

Although the mixed diamine macrocycles **72** and **79** showed to be equally difficult to analyse with NMR-spectroscopy as macrocycles **37** and **43**, the synthesis of mixed diamine macrocycles **72** and **79** proceeded essentially as well as the synthesis of bis-dinitrodianilines **37**. In other words, the synthetic procedures described in earlier chapters may be successfully employed in the preparation of a more diverse range of macrocycles. The stability issues observed with rigid macrocycles may be rectified by incorporation of a flexible diamine. As the diamines **9** and **65-69** only represent a small fragment of the aromatic diamines available the cyclophane **8** may be diversified into a virtually infinite number of macrocycles.

6: Concluding remarks

The imperative question of this thesis was how cyclophane **8** would perform as a host in molecular recognition with bioactive guest molecules. Unfortunately, the sheer number of encumbrances and pitfalls in the development of the synthesis of **8** has left this question unanswered. Judging from the stacked conformer **C2**, some kind of binding affinity towards smaller aromatic guests would however not be completely surprising.

As the deprotection of both bis-nitroanilines **37** and bis-(thio)ureas **43** failed, no binding studies could be performed with these cyclophanes in aqueous solution. Some preliminary attempts on binding studies of the protected cyclophanes in organic solvents were however performed.[91] Both **37** and **43** yielded ^{1}H-NMR-spectra of such poor resolution that the use of ^{1}H-NMR-titrations were deemed impossible. Furthermore, macrocycles **37** and **43** showed no detectable fluorescence, which excluded the use of fluorescence titrations. Therefore, UV-visual spectroscopy seemed to be the most promising method for determination of binding constants. Initial experiments with the host **37a** and the guest 4-nitrophenol in the solvent mixture $CHCl_3$/EtOH (50/1) unfortunately showed overlapping absorption signals of both host and guest so that no reliable binding constant could be established. The problem of overlapping signals could possibly be solved by employing a competitive binding study with UV-Vis absorbing substances, such as HABA **80** or Magneson **81** (Figure 6-1) as competitive probes.[92]



80 **81**

Figure 6-1: Probes for competitive binding studies.

So, what scientifical value could possibly be denoted to a thesis which hardly even tries to give an answer to the fundamental question defined in the introduction? The

answer to this self-critical question may be found in the somewhat audacious title of this thesis, namely "Flexible Synthesis of Rigid Cyclophanes". The major result of the scientific endeavours documented on these pages is the development of a versatile synthetical procedure which allows for preparation of a multitude of potential host molecules. More specifically, the following accomplishments could be considered as small but significant breakthroughs in this particular thesis:

- Synthesis of the diamine **9** hitherto overlooked in organic synthesis
- Application of **9** as a dinucleophile
- First example of the linker **30** being used for synthesis of macrocyclic dinitrodianilines
- Evaluation of carbamate reactivity toward nitrogen nucleophiles in macrocyclisations
- Application of this carbamate reactivity into synthesis of both bis-ureas and bis-thioureas
- Development of methods for the purification and analysis of macrocycles
- Diversification of synthesis with diamines **65** and **67**

The most obvious starting point for future work, apart from binding studies with the macrocycles at hand, is the synthesis of macrocycles **37** with amino acid side chains protected with *t*-butyl-groups. By using a mild acidolytic protocol these protective groups might very well be removed without disrupting the macrocyclic core structure and yield the elusive water-soluble cyclophanes. Although the deprotection of *t*-butyl protected bis-ureas **43** failed, the bis-nitroanilines **37** could be expected to be a bit more tolerant to acidolytic treatment, as the cyclophanes **37** have shown to be more stable upon storage.

Next, the synthesis of cyclophanes should be diversified with respect to the amino acids used. The syntheses documented here were largely focused on neutral and acidic side chains, thus overlooking interesting amino acids such as arginine and histidine. The neutral lipophilic amino acids, *e.g.* phenylalanine and leucine, may not be very useful in the synthesis of water-soluble cyclophanes but should not be completely ignored as cyclophanes incorporating these amino acids could be used as hosts in organic solutions.

Another point of future work would be the preparation of hybrid macrocycles with two different aromatic diamines. As noted in the end of chapter 4, the mixed bis-ureas **64** might show to be an ideal compromise between conformational flexibility and rigidity. The synthesis of a mixed bis-(thio)urea incorporating diamines **9** and **65** is a most challenging target, the mixed bis-(thio)urea with diamines **9** and **66** might be a more realistic target as the diamine **66** already is preorganised in a slightly bent structure.

Next point of future development could be the investigation of other linkers than the dinitrodianiline and the (thio)urea. Virtually any kind of dielectrophile with reactivity towards nitrogen nucleophiles could be employed, a few suggestions are included below (Figure 6-2).

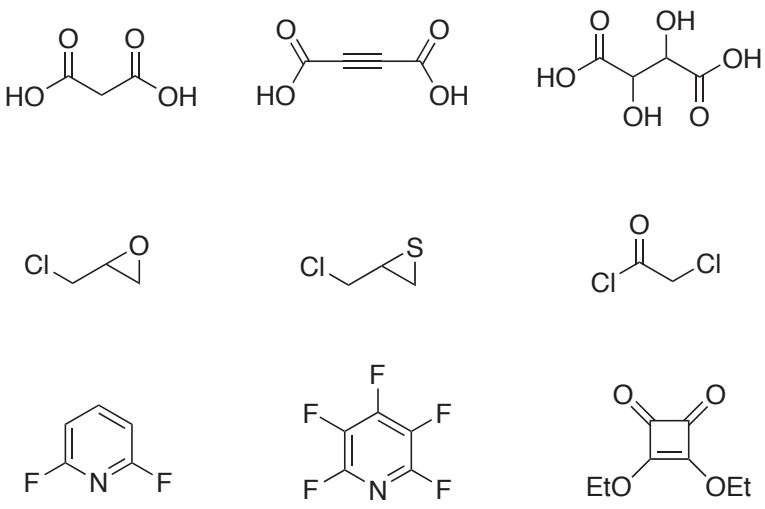
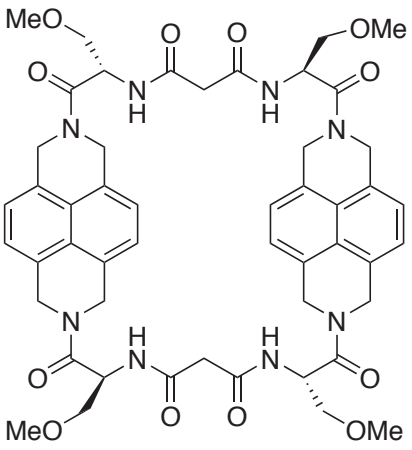
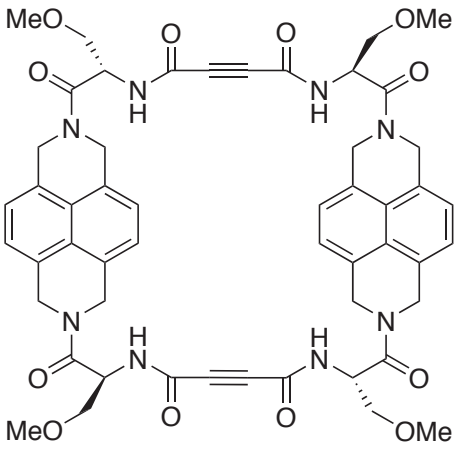


Figure 6-2: Potential linkers?

Out of curiosity, the first two of these linkers, namely malonic acid and acetylene dicarboxylic acid, were employed in conformational analyses. Once again, Monte Carlo simulations were perfomed with the program MacroModel, based on the force field MMFF94s with chloroform as solvent.



82



83

Figure 6-3: Structures subject to conformational analysis.

The flexible bis-malonic derivative **82** yielded 905 different conformers with an energy within 5 kcal/mol relative to the lowest energy conformer **C9** as seen from two different angles in Figure 6-4. As expected, the flexible linker resulted in a wide range of conformers with different orientation of the aromatic diamines and the malonic amides. It should be noted that the conformers with an energy close to **C9** also showed a conformational diversity. One striking example is the open cavity conformer **C10**, which was calculated to have an energy only 0.04 kcal/mol higher than **C9**.

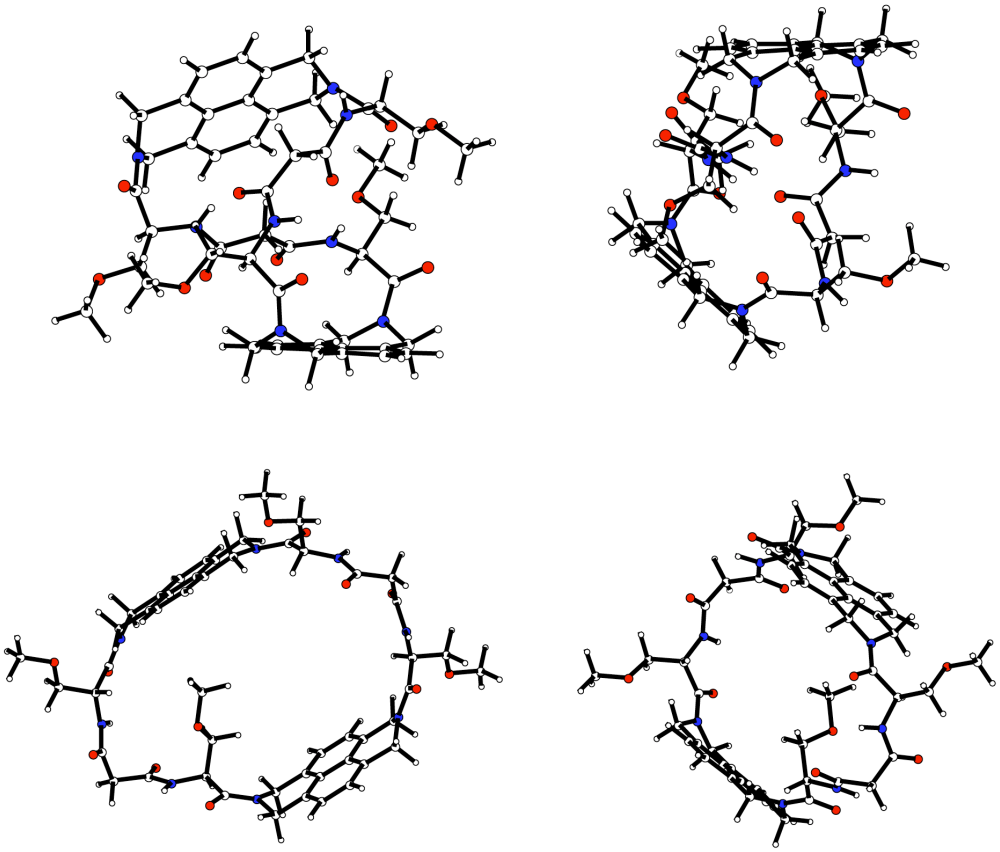


Figure 6-4: Top left, right: lowest energy conformer **C9**. Bottom left, right: ring conformer **C10**.

The more rigid bis-acetylenic derivative **83** yielded only 6 different conformers with an energy within 5 kcal/mol relative to the lowest energy conformer **C11** as seen from two different angles in Figure 6-5. The other low-energy conformers consisted of small variations in the orientation of the amino acid side chains and the acetylenic linkers. The conformer **C11** is remarkably similar to **C9** with respect to the crossed linker chains. An analogue of the open conformer **C10** was also found within the bis-acetylenic conformers, though this conformer **C12** was 6.2 kcal/mol higher in energy than **C11**.

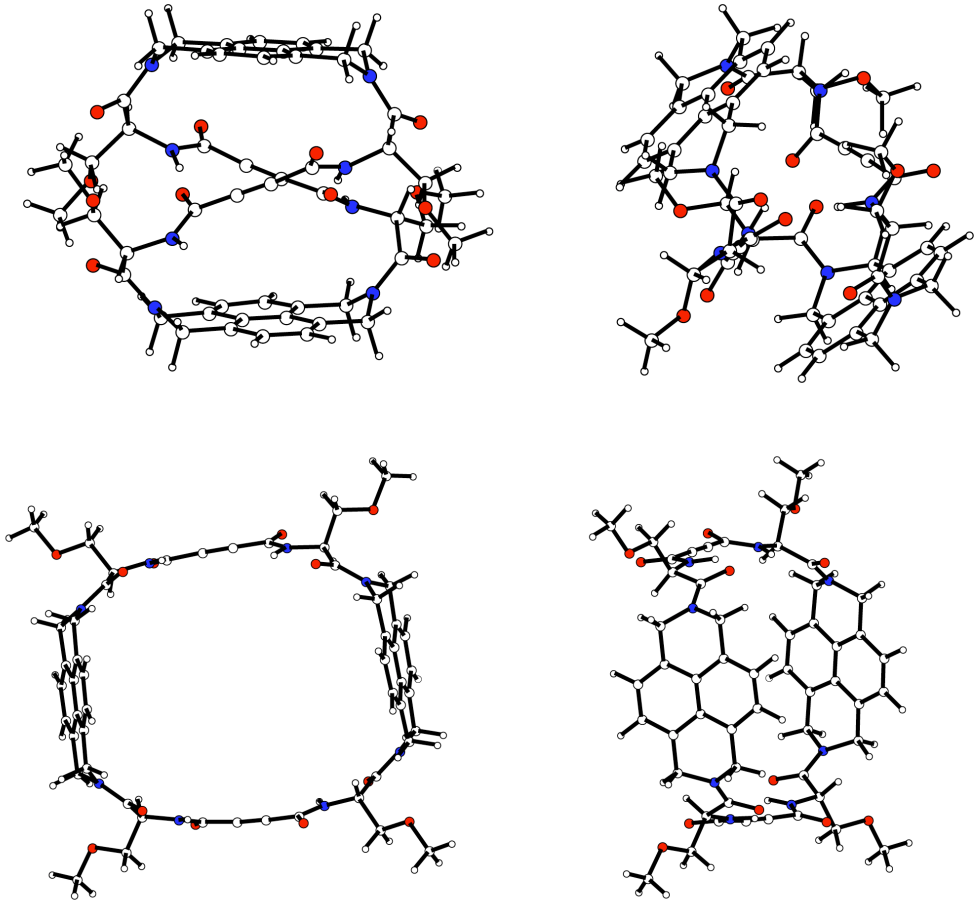


Figure 6-5: Top left, right: low energy conformer **C11**. Bottom left, right: ring conformer **C12**.

The distances between the aromatic diamines were measured as described in chapter 3 and are summarised below (Table 6-1). Based on the results of these conformational analyses, the bis-malonic amide macrocycle **82** is probably a more rewarding target molecule than **83**, as **82** readily transforms into a conformer **C10** with an open binding cavity easily accessible for a presumptive guest molecule.

Table 6-1: Measured distances in conformers **C9-C12**.

Conformer	d_1 (Å)	d_2 (Å)	d_3 (Å)
C9	9.8	9.5	8.8
C10	9.1	8.8	9.9
C11	9.7	9.7	8.2
C12	11.2	12.0	12.8

With all of these loose ends and possibilities available for future exploration and exploitation, this thesis should not be disparaged as a dead end in science, it should rather be considered as the first step toward preparation and application of a wide range of cyclophanes.

7: Acknowledgements

Nu ser jag äntligen ljuset i slutet av tunneln - och den här gången visar det sig inte vara ett mötande tåg. Det är därför dags att skriva några tacksamhetens ord till de personer utan vars hjälp jag fortfarande irrat runt i mörkret:

- Min handledare Ulf Nilsson, vars outsinliga optimism och gemyt är ett föredöme för oss alla.
- Professor Olov Sterner samt docenterna Ulf Ellervik och Ulf Lindström för att ha skapat en kreativ och positiv arbetsmiljö på avdelningen för bioorganisk kemi, fd organisk kemi 2.
- Maria Levin som har sett till att avdelningen VERKLIGEN har fungerat.
- Nuvarande och dåvarande skrivrumskamrater (Hans Grundberg, Pernilla Sörme, Johan Billing, Chris McKay, Christopher Öberg, Jenny Ågren, Kambiz Katebzadeh) som sett till att hålla produktiviteten på en lagom nivå.
- Labkamraterna (Johan Eriksson Bajtner, Yanhong He, Sumita Bhattacharyya, Bader Salameh, Patricia Mollinedo, Yonny Flores, Paul Okechukwu) som gjort de dagliga göromålen betydligt trivsammare.
- Alla medlemmar i grupp UN som kommit och gått under årens lopp för givande gruppmöten, både officiella och inofficiella.
- Karl-Erik Bergquist (NMR) och Einar Nilsson (MS) för ovärderlig hjälp med att lösa analytiska bekymmer.
- Anders Sundin som tagit hand om trilskande datorer.
- Alla övriga arbetskamrater (ingen nämnd - ingen glömd) under min tid på ok2 och bioorganisk kemi för att ha gjort arbetsdagarna så mycket behagligare, både inuti och utanför fikarummet.
- Avdelningen för organisk farmaceutisk kemi samt avdelningen för organisk kemi vid Uppsala universitet för en stimulerande introduktion till organisk kemi.
- GLIBS som betalade för en resa till en konferens i Kanada, Johan Billing som gjorde resan roligare.
- Folket utanför Kemicentrum (Carina, Marcus, Mariann, Melcer, Robert) som sett till att livet inte enbart handlat om organisk kemi.

- Folket i Arendal (Gull-Britt, Lars-Eric, Kicki, Niklas och Matilda) för en bakgrund och en framtid.

Avslutningsvis vill jag rikta ett tack till följande som medvetet eller omedvetet stått för moraliskt stöd och andlig vägledning: Svenska hembryggareföreningen, Lemmy Kilmeister, Jagged Alliance 2, Udo Dirkschneider, Bear´s Pit, P3, X-Com, Debbie Harry, Piled higher & Deeper, The Glimmer Twins, [58-08-2], Tom Sharpe, Sweden Rock Magazine, Nick Hornby, Lemon64, David Bowie, Humulus Lupulus, Terry Pratchett, Spinal Tap, SIDplayer, Tremolo Beer Gut, Groo, Lise Myhre, CCS64, Trocadero samt Järnjungfrun (S. Harris et al).

8: Supplementary data

2,3-Dihydro-1H-benzo[de]isoquinoline (19).
1,8-Naphthalimide (200 mg, 1.01 mmol) was suspended in dry THF (5 mL) and 1M BH_3*THF (3.0 mL, 3.0 mmol) were added while stirring. The reaction mixture was refluxed for 14 hours, then cooled to 0°C and quenched with CH_3OH (2 mL). (CAUTION: Excessive foaming may occur at this moment). 6M HCl (1 mL) was added and the mixture was refluxed another 2 hours before addition of 6M KOH (10 mL). After stirring 1 hour at room temperature the mixture was diluted with Et_2O (10 mL) and filtered, the filterbed was repeatedly extracted with Et_2O. The combined organic extracts were washed with water, dried over $MgSO_4$ and evaporated to give the crude product **19** as a greenish mass (118 mg, 67%). ^{1}H NMR($CDCl_3$): δ 7.70 (m, 2H), 7.37 (m, 2H), 7.18 (m, 2H), 4.33 (s, 4H).

***N,N*-Bis(2-propenyl)-2,7-diaza-1,3,6,8-tetrahydropyrene (21).**
Aluminium trichloride (9.25 g, 69 mmol) was dissolved in dry THF (250 mL) in an oven-dried 500-mL flask immersed in an ice-bath. Lithium aluminium hydride (7.80 g, 205 mmol) was slowly added and the mixture was stirred for 5 minutes. *N,N`*-Bis-(2-propenyl)-1,4,5,8-naphthalenetetracarboxylic diimide[37] **7** (10.0 g, 29 mmol) was then added and the resulting mixture was stirred at ambient temperature for 30 minutes before refluxing for 4 hours. The reaction was cooled to 0°C and cautiously poured on a slurry of ice (~300 g) and 2M KOH (200 mL). The solid was filtered and THF was evaporated off the filtered liquid, and the remaining aqueous phase was extracted with CH_2Cl_2 (200 mL). The filterbed was twice suspended in and filtered from CH_2Cl_2. The combined organic extracts (~600 mL) were washed with 2M KOH (300 mL), dried over CaH_2 and evaporated. The product was purified through flash chromatography on silica eluted with EtOAc/CH_2Cl_2/Et_3N (gradient from 18/2/1 to 10/10/1) and was obtained as pale solids, 6.3 g (75%). ^{1}H NMR ($CDCl_3$): δ 7.14 (s, 4H), 6.00 (m, 2H), 5.25 (m, 4H), 3.94 (s, 8H), 3.26 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 135.4, 131.5, 127.9, 121.9, 118.3, 60.5, 56.4. TLC (EtOAc/CH_3OH/Et_3N 16/4/1) R_f 0.65. EI-HRMS *m/z* calcd for $C_{20}H_{22}N_2$ $[M]^+$ 290.1783, found 290.1773.

***N,N*-Bis(ethoxycarbonyl)-2,7-diaza-1,3,6,8-tetrahydropyrene (22)**.
N,N-Bis(2-propenyl)-2,7-diaza-1,3,6,8-tetrahydropyrene **21** (5.29 g, 18 mmol) was suspended in dry THF (100 mL) and ethyl chloroformate (26 mL, 270 mmol) was added. The mixture was refluxed for 22 hours and evaporated into a brown mass. The crude product was crystallised from 75 mL CH_3OH/H_2O (4/1) and obtained as a pale powder, 4.45 g (70%).^{1}H NMR ($CDCl_3$): δ 7.23 (s, 4H), 4.90 (s, 8H), 4.20 (q, *J*=7 Hz, 4H), 1.27 (t, *J*=7 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 156.18, 130.79, 128.03, 122.14, 62.13, 47.05, 15.13; TLC (Heptane/EtOAc 1/1) R_f 0.50. FAB-HRMS *m/z* calcd for $C_{20}H_{23}N_2O_4$ $[M+H]^+$ 355.1659, found 355.1667.

2,7-Diaza-1,2,3,6,7,8-hexahydropyrene (9).
N,N-Bis(ethoxycarbonyl)-2,7-diaza-1,3,6,8-tetrahydropyrene **22** (1.00g, 2.8 mmol) was suspended in a mixture of dioxane (60 mL) and 2M KOH (120 mL) and refluxed for 24 hours. The dioxane was evaporated off and the remaining aqueous phase was extracted twice with CH_2Cl_2. The combined organic extracts were dried over CaH_2

and evaporated. The residue was purified by flash chromatography on silica (CH_2Cl_2/CH_3OH/Et_3N 20/0/1→20/1/1→20/2/1) and gave **3** as off-white solids, 0.47 g (80%). ^{1}H NMR ($CDCl_3$): δ 7.15 (s, 4H), 4.20 (s, 8H). TLC (EtOAc/CH_3OH/Et_3N 16:4:1) R_f 0.2. EI-HRMS *m/z* calcd for $C_{14}H_{14}N_2$ $[M]^+$ 210.1157, found 210.1150.

Typical acylation of 9: [S,S]-*N,N´*-Bis-[3-benzyloxy-2-(*N-t*-butoxycarbonyl-amino)-propionyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (17e).
2,7-Diaza-1,2,3,6,7,8-hexahydropyrene **9** (0.60 g, 2.9 mmol) was suspended and sonicated for 5 minutes at ambient temperature in $CHCl_3$ (150 mL, purified on basic aluminum oxide). To the sonicated suspension were added Boc-Ser(OBn)-OH (2.53 g, 8.6 mmol), EDC (2.19 g, 11.4 mmol), HOBt (0.88 g, 5.7 mmol), DMAP (1.44 g, 11.4 mmol) and $NaHCO_3$ (0.96 g, 11.4 mmol) and the resulting mixture was stirred at ambient temperature for 18 hours. The reaction was transferred to a separation funnel and washed with 5% HCl (2*200 mL), saturated $NaHCO_3$ (3*200 mL) and brine (3*200 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated into a pale solid (2.5 g), which was purified by flash chromatography (SiO_2, heptane/EtOAc 1:1) to give **17e** as off-white solids, 1.57 g (72%). A sample of analytical purity was prepared through precipitation from CH_3OH/H_2O (9:1).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30-7.00 (m, 14H), 5.50 (d, 2H, *J* = 8 Hz), 5.17-4.92 (m, 10H), 4.37 (s, 4H), 3.67-3.52 (m, 4H), 1.41 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.42, 155.34, 137.69, 130.45, 130.20, 128.38, 127.70, 127.56, 122.69, 122.50, 122.07, 121.83, 80.04, 77.43, 73.30, 71.18, 50.45, 48.90, 45.36, 28.52. TLC (heptane/EtOAc 1:1) R_f 0.25. $[\alpha]_D^{23}$ -3.9° (*c* 1, CH_2Cl_2). FAB-HRMS *m/z* calcd for $C_{44}H_{53}N_4O_8$ $[M+H]^+$ 765.3864, found 765.3859.

In similar manner were prepared:
***N,N´*-Bis-[2-(*N-t*-butoxycarbonylamino)-acetyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (17a).**
^{1}H NMR ($CDCl_3$): δ 7.36-7.25 (m, 4H), 5.54 (br, 2H), 5.12-5.09 (s, 4H), 4.88-4.85 (s, 4H), 4.17 (d, 4H, *J* = 7 Hz), 1.48 (s, 18H). TLC (CH_2Cl_2/CH_3OH 20:1) R_f 0.73. FAB-HRMS *m/z* calcd for $C_{28}H_{36}N_4O_6Na$ $[M+Na]^+$ 547.2533, found 546.2539.

[S,S]-*N,N´*-Bis-[2-(*N-t*-butoxycarbonylamino)-propionyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (17b).
^{1}H NMR ($CDCl_3$): δ 7.31-7.19 (m, 4H), 5.54 (d, 2H, *J* = 7 Hz), 5.21-5.10 (m, 2H), 4.97-4.80 (m, 8H), 1.42 (s, 18H), 1.26 (d, 6H, J = 7Hz). TLC (heptane/EtOAc 1:1) R_f 0.10. $[\alpha]^{21}_D$ - 15.6° (c1, CH_2Cl_2). FAB-HRMS *m/z* calcd for $C_{30}H_{41}N_4O_6$ $[M+H]^+$ 553.3027, found 553.3047.

[S,S]-*N,N´*-Bis-[2-(*N-t*-butoxycarbonylamino)-5-(2,3-dibenzyloxycarbonyl-guanidino)-pentanoyl]- 2,7-diaza-1,2,3,6,7,8-hexahydropyrene (17f).
^{1}H NMR ($CDCl_3$): δ 9.48-9.30 (br, 2H), 9.20-9.05 (br, 2H), 7.42-6.94 (m, 24H), 5.48-5.32 (dd br, 2H), 5.23-4.70 (m, 18H), 3.92-3.71 (br, 4H), 1.76-1.27 (br m 8H), 1.43 (s, 18H). TLC (heptane/EtOAc 1/1) R_f 0.26. $[\alpha]^{21}_D$ -3.0° (c1, CH_2Cl_2). FAB-HRMS *m/z* calcd for $C_{68}H_{79}N_{10}O_{14}$ $[M+H]^+$ 1259.5778, found 1259.5798.

[S,S]-*N,N´*-Bis-[6-(*N*-benzyloxycarbonylamino)-2-(*N-t*-butoxycarbonylamino)-hexanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (17g).

^{1}H NMR ($CDCl_3$): δ 7.40-7.20 (m, 14H), 5.45 (m, 2H), 5.20-4.68 (m, 16H), 3.15-2.90 (br, 4H), 1.74-1.20 (br m 12H), 1.41 (s, 18H). TLC (heptane/EtOAc 1/1) R_f 0.23. $[\alpha]^{23}_D$ +7.1° (c1, CH_2Cl_2). FAB-HRMS *m/z* calcd for $C_{52}H_{66}N_6O_{10}Na$ $[M+Na]^+$ 957.4738, found 957.4730.

[S,S]-*N,N´*-Bis-[2-(*N*-benzyloxycarbonylamino)-4-(*t*-butoxycarbonyl)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (17h).
^{1}H NMR ($CDCl_3$): δ 7.42-7.29 (m, 14H), 5.81 (m, 2H), 5.35-4.85 (m, 14H), 2.49-2.39 (br, 2H), 2.33-2.24 (br, 2H), 2.04-1.94 (br, 2H), 1.65-1.50 (br, 2H), 1.53 (s, 18H). TLC (heptane/EtOAc 1/1) R_f 0.27. $[\alpha]^{21}_D$ +33.7° (c1, CH_2Cl_2). FAB-HRMS *m/z* calcd for $C_{48}H_{56}N_4O_{10}Na$ $[M+Na]^+$ 871.3894, found 871.3876.

[S,S]-*N,N´*-Bis-[2-(*N*-benzyloxycarbonylamino)-6-(*N-t*-butoxycarbonylamino)-hexanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (17i).
^{1}H NMR ($CDCl_3$): δ7.35-7.00 (m, 14H), 5.80-5.67 (br, 2H), 5.10-4.40 (m, 16H), 2.90-2.75 (br, 4H), 1.65-1.35 (br m, 30H). TLC (heptane/EtOAc 1/2) R_f 0.19.

Synthesis of 23 - General method for removal of Boc with TFA.
Compound **17c** (200 mg, 0.235 mmol) was dissolved in CH_2Cl_2 (10 mL). To this solution were added Et_3SiH (0.150 mL, 0.94 mmol) followed by TFA (0.525 mL, 7.1 mmol) and the solution was stirred at room temperature overnight before evaporation into a pale syrup. The crude product **23c•TFA** was used immediately without any further purification.

Synthesis of 23 - General method for removal of Boc with HCl•Et_2O.
Compound **17c** (100 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (3 mL). To this solution were added Et_3SiH (0.075 mL, 0.47 mmol) followed by 2M HCl*Et_2O (2 mL) and the solution was stirred at room temperature overnight before evaporation into a pale mass. The crude product **23c•HCl** was used immediately without any further purification.

Synthesis of 23 - General method for removal of Z with ammonium formate.
Compound **17h** (100 mg, 0.12 mmol) was dissolved in THF (5 mL). To this solution were added ammonium formate (100 mg, 1.6 mmol) and 50 mg Pd/C (10%). The resulting mixture was stirred at room temperature overnight and then filtered. The filterbed was rinsed repeatedly with THF and CH_3OH and the combined solvents were evaporated into a pale mass. The crude product **23h** was used immediately without any further purification.

***N,N´*-Bis-acetyl-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (24).**
^{1}H NMR ($CDCl_3$): δ7.32-7.24 (m, 4H), 5.06 (s, 4H), 4.90 (s, 4H), 2.24 (s, 6H). FAB-HRMS *m/z* calcd for $C_{18}H_{19}N_2O_2$ $[M+H]^+$ calc 295.1447, found 295.1448.

***N*-Benzyloxycarbonyl-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (25).**
2,7-Diaza-1,2,3,6,7,8-hexahydropyrene **9** (130 mg, 0.62 mmol) and Cs_2CO_3 (300 mg, 0.93 mmol) were suspended in a mixture of CH_2Cl_2 (50 mL) and THF (15 mL). To this suspension was added a solution of benzyl chloroformate (0.084 mL, 0.59 mmol) in CH_2Cl_2 (10 mL) over a period of 30 minutes. The resulting mixture was stirred at room temperature for 17 hours, evaporated into a pale mass and purified by flash chromatography on silica (CH_2Cl_2/CH_3OH/Et_3N 80/2/4→80/3/4) to give **25** as a

greenish mass, 44 mg (19%). ^{1}H NMR ($CDCl_3$): δ 7.45-7.10 (m, 9H), 5.18 (s, 2H), 4.95 (s, 4H), 4.28 (s, 4H). TLC (EtOAc/CH_3OH/Et_3N 16/4/1) R_f 0.52.

_N_-Benzyloxycarbonyl-_N´_-_t_-butoxycarbonyl-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (26).

_N_-Benzyloxycarbonyl-2,7-diaza-1,2,3,6,7,8-hexahydropyrene **25** (40 mg, 0.12 mmol) was suspended in THF (10 mL) together with Cs_2CO_3 (76 mg, 0.24 mmol), DMAP (29 mg, 0.24 mmol) and Boc_2O (51 mg, 0.24 mmol) and stirred at room temperature for 20 hours, evaporated into a pale mass and purified by flash chromatography on silica (heptane/EtOAc 1/1) to give **13** as white solids, 19 mg (36%). ^{1}H NMR ($CDCl_3$): δ 7.38-7.21 (m, 9H), 5.17 (s, 2H), 4.95 (s, 4H), 4.87 (s, 4H), 1.48 (s, 9H). TLC (heptane/EtOAc 1/1) R_f 0.74. ES-HRMS _m/z_ calcd for $C_{27}H_{28}N_2O_4Na$ $[M+Na]^+$ calc 467.1947, found 467.1939.

[S]-Dimethyl-_N_-(2,4-dinitro-5-fluorophenyl)-glutamate (34).

1,5-Difluoro-2,4-dinitrobenzene **30** (25 mg, 0.12 mmol) was suspended in THF (5 mL) together with $NaHCO_3$ (20 mg, 0.19 mg) and L-glutamic acid dimethyl ester hydrochloride (30 mg, 0.14 mg). The mixture was stirred at ambient temperature for 2 hours, then evaporated and purified by flash chromatography on silica (heptane/EtOAc 1/1) to give **34** as a yellow oil, 35 mg (82%). %). ^{1}H NMR ($CDCl_3$): δ 9.15 (d, _J_=9 Hz, 1H), 8.95 (br d,1H), 6.74 (d, _J_=13 Hz, 1H), 4.43 (m, 1H), 2.52 (m, 2H), 2.27 (m, 2H). TLC (heptane/EtOAc 1/1) R_f 0.57.

[S,S]-Bis-_N,N´_-(dimethylglutamyl)-5-amino-2,4-dinitroaniline (35).

Compound **34** (35 mg, 0.098 mmol) was dissolved in THF (5 mL) and to this solution were added $NaHCO_3$ (20 mg, 0.19 mmol) and glutamic acid dimethyl ester hydrochloride (25 mg, 0.12 mmol). The mixture was refluxed for 23 hours, then evaporated and purified by flash chromatography on silica (heptane/EtOAc 1/1→1/4) to give **35** as a yellow oil, 38 mg (76%). ^{1}H NMR ($CDCl_3$): δ 9.23 (s, 1H), 8.83 (br d, 2H), 6.07 (s, 1H), 4.45 (m, 2H), 2.52 (m, 4H), 2.25 (m, 4H). TLC (heptane/EtOAc 1/1) R_f 0.24.

4-Chlorophenyl 4-nitrophenyl carbonate (50).

4-Chlorophenol (0.69 g, 5.4 mmol) and 4-nitrophenyl chloroformate (1.13 g, 5.6 mmol) were dissolved in dry CH_2Cl_2 (90 mL) and cooled to 0°C before dropwise addition of Et_3N (0.75 mL, 5.4 mmol) dissolved in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 2 hours, then washed with water (2*50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$ and evaporated into the product **50** as white solids, 1.65 g (97%). ^{1}H NMR ($CDCl_3$): δ 8.37 (d, 2H, _J_ = 8 Hz), 7.55 (d, 2H, _J_ = 8 Hz), 7.45 (d, 2H, _J_ = 8 Hz), 7.30 (d, 2H, _J_ = 8Hz).

3-Nitrophenyl 4-nitrophenyl carbonate (51).

3-Nitrophenol (0.69 g, 5.4 mmol) and 4-nitrophenyl chloroformate (1.13 g, 5.6 mmol) were dissolved in dry CH_2Cl_2 (90 mL) and cooled to 0°C before dropwise addition of Et_3N (0.75 mL, 5.4 mmol) dissolved in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 2 hours, then washed with water (2*50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$ and evaporated into the product **51** as pale solids, 1.56 g (95%). ^{1}H NMR ($CDCl_3$): δ 8.45 (m, 2H), 8.32 (m, 2H), 7.72

(m, 2H), 7.62 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 155.32, 151.19, 150.90, 149.30, 146.30, 130.94, 127.55, 125.97, 122.10, 121.73, 117.13.

4-Cyanophenyl chloroformate (54).
4-Cyanophenol (1.72 g, 14.4 mmol) was suspended in dry CH_2Cl_2 (50 mL) and cooled to 0°C. Diphosgene (2.39 mL, 19.8 mmol) (CAUTION: Diphosgene is a toxic lachrymator; all parts of this experiment, including evaporation, should be performed in a well ventilated hood) was slowly added followed by N-ethyldiisopropylamine (2.47 mL, 14.4 mmol). The reaction was stirred at 0°C for 2 hours, at ambient temperature overnight, and finally refluxed for 2 hours before evaporation. The residue was suspended in dry THF (15 mL) and the white precipitate was filtered off. The mother liquor was concentrated into a crude product (2.94 g) contaminated with approximately 10% of 4-cyanophenol and N-ethyldiisopropylamine hydrochloride. No further purfication was done. ^{1}H NMR($CDCl_3$): δ 7.70 (d, 2H, *J* = 8 Hz), 7.35 (d, 2H, *J* = 8 Hz).

4-Methoxycarbonylphenyl chlorothionoformate (55).
Methyl 4-hydroxybenzoate (2.20 g, 14.4 mmol) was suspended in dry CH_2Cl_2 (60 mL) and cooled to 0°C. Thiophosgene (1.51 mL, 19.8 mmol) (CAUTION: Thiophosgene is a toxic lachrymator; all parts of this experiment, including evaporation, should be performed in a well ventilated hood) was slowly added followed by N-ethyldiisopropylamine (2.47 mL, 14.4 mmol). The reaction was stirred at 0°C for 2 hours, at ambient temperature overnight, and finally refluxed for 2 hours before evaporation. The residue was suspended in dry THF (30 mL) and the white precipitate was filtered off. The mother liquor was concentrated into a crude product (4.14 g) contaminated with approximately 20% of methyl 4-hydroxybenzoate and N-ethyldiisopropylamine hydrochloride. No further purfication was done. ^{1}H NMR ($CDCl_3$): δ 8.15 (d, 2H, *J* = 6 Hz), 7.20 (d, 2H, *J* = 6 Hz), 3.90 (s, 3H).

Typical synthesis of an active carbamate: [S,S]-*N,N´*-Bis-[4-(benzyloxycarbonyl)-2-(*N*-4-methoxycarbonylphenoxycarbonylamino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (52h).
Compound **17c** (200 mg, 0.235 mmol) was dissolved in CH_2Cl_2 (10 mL) and triethylsilane (150 μL, 0.94 mmol) was added followed by TFA (525 μL, 7.1 mmol). The solution was stirred at ambient temperature for 16 hours and then evaporated into a yellow syrup. This syrup was dissolved in dry THF (15 mL) and Cs_2CO_3 (460 mg, 1.41 mmol) and 4-methoxycarbonylphenyl chloroformate (303 mg, 1.41 mmol) were added. The resulting suspension was stirred at ambient temperature for 3 hours and then evaporated. Flash chromatography (SiO_2, heptane/EtOAc 1:1 → 1:2) yielded product **53h** as a white solid (170 mg, 72%). ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 4H, *J* = 6 Hz), 7.45-7.25 (m, 14H), 7.15 (d, 4H, *J* = 6 Hz), 6.40 (d, 2H, *J* = 7 Hz), 5.35-4.80 (m, 14H), 3.87 (s, 6H), 2.75-2.60 (br, 2H), 2.55-2.40 (br, 2H), 2.20-2.05 (br, 2H), 1.75-1.60 (br, 2H). TLC (heptane/EtOAc 1:1) R_f 0.07. MS (MALDI-TOF) $[M+H]^+$ calc 1005.4, found 1005.6, $[M+Na]^+$ calc 1027.3, found 1027.4. In general, the carbamates and thiocarbamates **53** were too unstable for HRMS (FAB). Furthermore, due to their inherent reactivity, spectroscopic analyses of compound **53** were difficult.

In a similar manner were prepared:

[S,S]-*N,N´*-Bis-[3-benzyloxy-2-(*N*-phenoxycarbonylamino)-propionyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53b).
^{1}H NMR ($CDCl_3$): δ 7.45-6.95 (m, 24H), 6.12 (d, 2H, *J* = 7 Hz), 5.25-4.80 (m, 10H), 4.40 (s, 4H), 3.75-3.50 (m, 4H). TLC (heptane/EtOAc 1:2) R_f 0.48. MS (MALDI-TOF) $[M+Na]^+$ calc 827.3, found 827.0.

[S,S]-*N,N´*-Bis-[4-(benzyloxycarbonyl)-2-(*N*-phenoxycarbonylamino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53c).
^{1}H NMR ($CDCl_3$): δ 7.40-7.00 (m, 24H), 6.18 (d, 2H, *J* = 8 Hz), 5.30-4.80 (m, 14H), 2.75-2.60 (br, 2H), 2.50-2.35 (br, 2H), 2.15-2.00 (br, 2H), 1.75-1.60 (br, 2H). MS (MALDI-TOF) $[M+H]^+$ calc 889.4, found 887.9, $[M+Na]^+$ calc 911.9, found 911.9.

[S,S]-*N,N´*-Bis-[3-benzyloxy-2-(*N*-4-fluorophenoxycarbonylamino)-propionyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53d).
^{1}H NMR ($CDCl_3$): δ 7.35-6.97 (m, 22H), 6.21 (d, 2H, *J* = 7 Hz), 5.22-4.90 (m, 14H), 4.37 (s, 4H), 3.76-3.60 (m, 4H). TLC (heptane/EtOAc 1:2) R_f 0.62. MS (MALDI-TOF) $[M+H]^+$ calc 841.3, found 843.9, $[M+Na]^+$ calc 863.3, found 864.0.

[S,S]-*N,N´*-Bis-[3-benzyloxy-2-(*N*-4-chlorophenoxycarbonylamino)-propionyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53e).
^{1}H NMR ($CDCl_3$): δ 7.35-6.95 (m, 22H), 6.30 (br, 2H), 5.21-4.85 (m, 14H), 4.37 (s, 4H), 3.76-3.60 (m, 4H); TLC (heptane/EtOAc 1:1) R_f 0.29.

[S,S]-*N,N´*-Bis-[4-(benzyloxycarbonyl)-2-(*N*-4-chlorophenoxycarbonylamino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53f).
^{1}H NMR ($CDCl_3$): δ 7.65 (d, 1H, *J* = 7 Hz), 7.43-7.25 (m, 18H), 7.03 (d, 2H, *J* = 6 Hz), 6.23 (d, 1H, *J* = 7 Hz), 5.35-4.83 (m, 14H), 2.71-2.57 (br, 2H), 2.53-2.40 (br, 2H), 2.18-2.02 (br, 2H), 1.76-1.62 (br, 2H). TLC (heptane/EtOAc 1:1) R_f 0.25. MS (MALDI-TOF) $[M+Na]^+$ calc 979.2, found 980.5.

[S,S]-*N,N´*-Bis-[3-(benzyloxycarbonyl)-2-(*N*-4-chlorophenoxycarbonylamino)-propionyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53g).
^{1}H NMR ($CDCl_3$): δ 7.45-6.90 (m, 22H), 5.35-4.50 (br m, 14H), 3.20-2.55 (br m, 4H). TLC (heptane/EtOAc 1:1) R_f 0.28.

[S,S]-*N,N´*-Bis-[4-(benzyloxycarbonyl)-2-(*N*-4-cyanophenoxycarbonylamino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53i).
^{1}H NMR ($CDCl_3$): δ 7.70-7.20 (m, 22H), 6.40 (d, 2H, *J* = 7 Hz), 5.40-4.85 (m, 14H), 2.75-2.40 (br, 4H), 2.20-2.05 (br, 2H), 1.80-1.60 (br, 2H). TLC (heptane/EtOAc 1:1) R_f 0.12. MS (MALDI-TOF) $[M+Cs]^+$ calc 1071.2, found 1071.9.

[S,S]-*N,N´*-Bis-[4-(benzyloxycarbonyl)-2-(*N*-4-chlorophenoxythiocarbonyl-amino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53j).
^{1}H NMR ($CDCl_3$): δ 7.97-7.86 (m, 2H), 7.30- 7.10 (m, 18H), 6.85 (d, 4H, *J* = 9 Hz), 5.65-5.55 (br, 2H), 5.26-4.72 (m, 12H), 2.64-2.52 (br, 2H), 2.40-2.30 (br, 2H), 2.17-2.05 (br, 2H), 1.77-1.68 (br, 2H). TLC (heptane/EtOAc 1:1) R_f 0.18. MS (MALDI-TOF) $[M+H]^+$ calc 989.2, found 991.6, $[M+Cs]^+$ calc 1121.1, found 1120.8.

[S,S]-*N,N´*-Bis-[4-(benzyloxycarbonyl)-2-(*N*-2,3,4,5,6-pentafluorophenoxy-thiocarbonylamino)-butanoyl]-1,2,3,6,7,8-hexahydro-2,7-diazapyrene (53k).

^{1}H NMR ($CDCl_3$): δ 7.32-7.17 (m, 14H), 5.23-4.74 (m, 14H), 2.64-2.53 (br, 2H), 2.52-2.38 (br, 2H), 2.07-1.93 (br, 2H), 1.93-1.78 (br, 2H). TLC (heptane/EtOAc 1:1) R_f 0.35.

[S,S]-*N*,*N*´-Bis-[4-(benzyloxycarbonyl)-2-(*N*-4-fluorophenoxycarbonylamino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53l).
^{1}H NMR ($CDCl_3$): δ 7.50-6.80 (m, 22H), 6.05 (br, 1H), 5.35-4.75 (m, 14H), 2.65-2.30 (br, 4H), 2.10-1.90 (br, 2H), 1.70-1.50 (br, 2H). TLC (heptane/EtOAc 1:1) R_f 0.27. MS (MALDI-TOF) $[M+H]^+$ calc 925.3, found 925.4, $[M+Na]^+$ calc 947.3, found 946.8.

[S,S]-*N*,*N*´-Bis-[4-(benzyloxycarbonyl)-2-(*N*-4-methoxycarbonylphenoxy-thiocarbonylamino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53m).
^{1}H NMR ($CDCl_3$): δ 8.15 (d, 4H, *J* = 6 Hz), 7.50-7.30 (m, 14H), 7.23 (d, 4H, *J* = 6 Hz), 5.45-4.90 (m, 14H), 4.00 (s, 6H), 2.87-2.53 (br, 4H), 2.45-2.00 (br, 2H), 2.00-1.76 (br, 2H). TLC (heptane/EtOAc 1:1) R_f 0.19.

[S,S]-*N*,*N*´-Bis-[3-(benzyloxycarbonyl)-2-(*N*-4-methoxycarbonylphenoxy-carbonylamino)-propionyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53n).
^{1}H NMR ($CDCl_3$): δ 7.97 (d, 2H, *J* = 6 Hz), 7.37-6.95 (m, 18H), 5.37-4.48 (br m, 14H), 3.15-2.65 (br m, 4H). TLC (heptane/EtOAc 1:1) R_f 0.23. MS (MALDI-TOF) $[M+H]^+$ calc 977.3, found 977.6, $[M+Na]^+$ calc 999.3, found 999.4.

[S,S]-*N*,*N*´-Bis-[3-(benzyloxycarbonyl)-2-(*N*-2,3,4,5,6-pentafluorophenoxy-thiocarbonylamino)-propionyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53o).
^{1}H NMR ($CDCl_3$): δ 7.45-7.25 (m, 14H), 5.37-4.48 (br m, 14H), 3.25-2.73 (br m, 4H). TLC (heptane/EtOAc 1:1) R_f 0.47.

[S,S]-*N*,*N*´-Bis-[6-(*N*-benzyloxycarbonylamino)-2-(*N*-4-methoxycarbonyl-phenoxycarbonylamino)-hexanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53p).
^{1}H NMR ($CDCl_3$): δ 7.92 (d, 4H, *J* = 8 Hz), 7.30-7.15 (m, 14H), 7.10 (d, 4H, *J* = 8 Hz), 6.25-6.10 (m, 2H), 5.20-4.60 (br m, 14H), 3,81 (s, 6H), 3.12-2.83 (br 4H), 1.75-1.25 (br m, 12H); TLC (heptane/EtOAc 1/2) R_f 0.23; MS (MALDI-TOF) $[M+Na]^+$ calc 1113.4, found 1115.4, $[M+Cs]^+$ calc 1223.3, found 1225.2.

[S,S]-*N*,*N*´-Bis-[4-(*t*-butyloxycarbonyl)-2-(*N*-4-methoxycarbonylphenoxy-carbonylamino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53q).
^{1}H NMR ($CDCl_3$): δ 8.04 (d, 4H, *J* = 8 Hz), 7.44-7.27 (m, 4H), 7.19 (d, 4H, *J* = 8 Hz), 6.21 (m, 2H), 5.40-4.89 (m, 10H), 3.92 (s, 6H), 2.55-2.28 (br, 4H), 2.10-1.95 (br, 2H), 1.73-1.60 (br, 2H), 1.49 (s, 18H). TLC (heptane/EtOAc 1/1) R_f 0.15. MS (MALDI-TOF) $[M+Na]^+$ calc 959.3, found 959.8, $[M+Cs]^+$ calc 1069.3, found 1069.5.

[S,S]-*N*,*N*´-Bis-[4-(benzyloxycarbonyl)-2-(*N*-3-trifluoromethylphenoxy-carbonylamino)-butanoyl]-2,7-diaza-1,2,3,6,7,8-hexahydropyrene (53r).
^{1}H NMR ($CDCl_3$): δ 7.51-7.27 (m, 22H), 6.24-6.15 (br, 2H), 5.36-4.85 (m, 14H), 2.74-2.42 (br, 4H), 2.16-2.01 (br, 2H), 1.75-1.62 (br, 2H). TLC (heptane/EtOAc 1/1) R_f 0.20. MS (MALDI-TOF) $[M+H]^+$ calc 1025.3, found 1024.0 $[M+Na]^+$ calc 1047.3, found 1047.7, $[M+Cs]^+$ calc 1157.2, found 1157.3.

Typical synthesis of a macrocyclic bis-urea or bis-thiourea: **Macrocycle 43f.**
Compound **17d** (131 mg, 0.159 mmol) was dissolved in CH_2Cl_2 (7 mL) and triethylsilane (100 μL, 0.63 mmol) was added followed by TFA (355 μL, 4.8 mmol). The solution was stirred at ambient temperature for 16 hours and then evaporated into a yellow syrup. This syrup was dissolved in dry THF (220 mL) to which were added Cs_2CO_3 (460 mg, 1.41 mmol) and compound **53h** (160 mg, 0.159 mmol). The resulting suspension was stirred at ambient temperature for 5 hours and then evaporated. The residue was dissolved in $CHCl_3$ (50 mL) and washed with 0.2M KOH (4*50 mL) followed by saturated NaCl (2*50 mL), dried over $MgSO_4$ and concentrated. Flash chromatography (SiO_2, toluene/CH_3OH 20:1 → 18:1 → 16:1) yielded **43f** as a pale solid (110 mg, 52%). TLC (toluene/CH_3OH 9:1) R_f 0.24. $[\alpha]^{23}_D$ +25.5° (c 0.2, CH_2Cl_2). MS (MALDI-TOF) $[M+H]^+$ calc 1321.5, found 1322.8, $[M+Na]^+$ calc 1343.5, found 1344.4. ES-HRMS *m/z* calcd for $C_{76}H_{73}N_8O_{14}$ $[M+H]^+$ calc 1321.5247, found 1321.5286.^{1}H NMR spectrum was poorly resolved with broad peaks; no assignment is included here.

In a similar manner were prepared:

Macrocycle 43a.
Compound **17e** and **53b** gave **43a** in 43% yield (refluxing conditions). TLC (CH_2Cl_2/CH_3OH 20:1) R_f 0.28. MS (MALDI-TOF) $[M+H]^+$ calc 1181.5 found 1183.2, $[M+Na]^+$ calc 1203.5, found 1205.3.

Macrocycle 43b.
Undetected by MALDI-TOF and TLC.

Macrocycle 43c.
MS (MALDI-TOF) $[M+H]^+$ calc 1265.5, found 1264.9, $[M+Na]^+$ calc 1287.5, found 1287.0.

Macrocycle 43d.
Compound **17c** and **53f** gave **43d** in 51% yield. TLC (toluene/CH_3OH 9:1) R_f 0.26. MS (MALDI-TOF) $[M+H]^+$ calc 1349.6, found 1350.9, $[M+Na]^+$ calc 1371.5, found 1372.3. ES-HRMS *m/z* calcd for $C_{78}H_{77}N_8O_{14}$ $[M+H]^+$ calc 1349.5560, found 1349.5581.

Macrocycle 43e.
Compound **17e** and **53g** gave **43e** in 55% yield. TLC (toluene/CH_3OH 9:1) R_f 0.16. MS (MALDI-TOF) $[M+H]^+$ calc 1237.5, found 1236.6, $[M+Na]^+$ calc 1259.5, found 1258.8.

Macrocycle 43g.
Compound **17d** and **53m** gave **43g** in 54% yield. TLC (toluene/CH_3OH 9:1) R_f 0.18. MS (MALDI-TOF) $[M+H]^+$ calc 1353.5, found 1354.3 $[M+Na]^+$ calc 1375.5, found 1375.5. ES-HRMS *m/z* calcd for $C_{76}H_{73}N_8O_{10}S_2$ $[M+H]^+$ calc 1353.4789, found 1353.4791.

Macrocycle 43h.
Compound **17c** and **53j** gave **43h** in 59% yield. TLC (toluene/CH_3OH 9:1) R_f 0.23. MS (MALDI-TOF) $[M+H]^+$ calc 1381.5, found 1382.5, $[M+Na]^+$ calc 1403.5, found

1403.5. ES-HRMS *m/z* calcd for $C_{78}H_{77}N_8O_{12}S_2$ $[M+H]^+$ calc 1381.5102, found 1381.5074.

Macrocycle 43i.
Compound **17e** and **53j** gave **43i** in 52% yield. TLC (toluene/CH_3OH 9:1) R_f 0.24. MS (MALDI-TOF) $[M+H]^+$ calc 1297.5 found 1298.2, $[M+Na]^+$ calc 1319.5, found 1320.1. ES-HRMS *m/z* calcd for $C_{74}H_{73}N_8O_{10}S_2$ $[M+H]^+$ calc 1297.4891, found 1297.4883.

Macrocycle 43j.
Compound **17a** and **53h** gave **43j** in 33% yield. TLC (toluene/CH_3OH 9:1) R_f 0.07. MS (MALDI-TOF) $[M+H]^+$ calc 1025.4, found 1026.7, $[M+Na]^+$ calc 1047.4, found 1048.4. ES-HRMS *m/z* calcd for $C_{58}H_{57}N_8O_{10}$ $[M+H]^+$ calc 1025.4918, found 1025.4202.

Macrocycle 43k.
Compound **17d** and **53n** gave **43k** in 61% yield. TLC (toluene/CH_3OH 9:1) R_f 0.18. MS (MALDI-TOF) $[M+H]^+$ calc 1293.5, found 1294.4, $[M+Na]^+$ calc 1315.5, found 1316.7.

Macrocycle 43l.
Compound **17d** and **53o** gave **43l** in 22% yield. TLC (toluene/CH_3OH 9:1) R_f 0.18. MS (MALDI-TOF) $[M+H]^+$ calc 1269.5, found 1270.1 $[M+Na]^+$ calc 1291.4, found 1292.1. ES-HRMS *m/z* calcd for $C_{72}H_{69}N_8O_{10}S_2$ $[M+H]^+$ calc 1269.4579, found 1269.4612.

Macrocycle 43m.
Undetected by MALDI-TOF and TLC.

Macrocycle 43n.
Compound **17h** and **53q** gave **43n** in 27% yield. TLC (toluene/CH_3OH 9:1) R_f 0.11. MS (MALDI-TOF) $[M+H]^+$ calc 1213.6, found 1214.6 , $[M+Na]^+$ calc 1235.6, found 1235.7.

[S,S]-*N,N´*-Bis-[3-benzyloxy-2-(*N-t*-butoxycarbonylamino)-propionyl]-*p*-xylylenediamine (70).
p-Xylylenediamine **67** (0.50 g, 3.67 mmol) was suspended in THF (100 mL) together with Boc-Ser(OBn)-OH (3.25 g, 11.0 mmol), DIC (3.45 mL, 22.0 mmol), HOBt (1.12 g, 7.34 mmol), DMAP (1.85 g, 14.7 mmol) and Cs_2CO_3 (4.78 g 14.7 mmol) and the resulting milky white suspension was stirred at room temperature for 18 hours followed by evaporation of most of the THF. The residue was diluted with $CHCl_3$ (100 mL) and washed with 5% HCl (2*150 mL), saturated $NaHCO_3$ (3*150 mL) and brine (3*150 mL). The organic phase was then dried over $MgSO_4$ and evaporated into off-white solids. This crude material was purified by flash chromatography on silica (heptane/EtOAc 1/1→1/2) to give 1.71 g of the product contaminated with diisopropylurea. Precipitation from CH_3OH (20 mL) gave pure product **70** as white solids, 0.88 g (35%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.35-7.22 (m, 10 H), 7.15 (s, 4H), 6.76 (m, 2H), 5.47-5.36 (br, 2H), 4.57-4.29 (m, 10H), 3.95 (m, 2H), 3.60 (m, 2H), 1.43 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.42, 155.73, 137.54, 137.36,

128.69, 128.15, 128.02, 127.95, 80.54, 73.66, 70.14, 54.29, 43.35, 28.46. TLC (heptane/EtOAc 1:2) R_f 0.45. $[\alpha]_D^{21}$ +28.8° (*c* 1, CH_2Cl_2). FAB-HRMS *m/z* calcd for $C_{38}H_{50}N_4O_8Na$ $[M+Na]^+$ calc 713.3527, found 713.3533.

Macrocycle 72.
Compound **70** (100 mg, 0.145 mmol) was dissolved in CH_2Cl_2 (7 mL). Triethylsilane (90 μL, 0.58 mmol) was added, followed by TFA (320 μL, 4.3 mmol). The solution was stirred at ambient temperature for 16 hours and then evaporated into a syrup. This syrup was dissolved in THF (150 mL, dried over MS) to which were added Cs_2CO_3 (283 mg, 0.87 mmol) and compound **36e** (135 mg, 0.145 mmol). The resulting suspension was refluxed for 20 hours and then evaporated. Flash chromatography (SiO_2, heptane/EtOAc 1/1→1/2) yielded **72** as a yellow solid, 17 mg (8.5%). ES-HRMS *m/z* calcd for $C_{74}H_{71}N_{12}O_{16}$ $[M+H]^+$ 1383.5111, found 1383.5105. TLC (heptane/EtOAc 1:4) R_f 0.73.

***N,N´*-Dibenzyl-2,3:6,7-anthracenedicarboximide (74).**
Octabromide **73** (5.51 g, 7.2 mmol) and *N*-benzylmaleimide (2.70 g, 14.4 mmol) were suspended in DMAc (60 mL) and the suspension was heated to 80°C before addition of NaI (10.8 g, 72 mmol). The resulting mixture was stirred at 80°C for 48 hours, then diluted with water (180 mL) and refluxed for 5 minutes before cooling to room temperature. The suspension was filtered and the bright yellow filterbed was washed repeatedly with water. The filtered solids were precipitated from acetone (80 mL) to give the product **74** as bright yellow solids, 2.0 g (56%). ^{1}H NMR (300 MHz, D_6-DMSO) showed no traces of starting materials in the product.

Bis-2,3:6,7-(*N*-benzyl-iminodimethylene)-anthracene (75).
Aluminium trichloride (1.61 g, 12.1 mmol) was suspended in THF (40 mL) in a three-necked round bottom flask immersed in an ice bath. $LiAlH_4$ (1.30 g, 34.3 mmol) was slowly added and the mixture was stirred at 0°C for 10 minutes before cautious addition of **74** (2.0 g, 4.0 mmol). The resulting suspension was stirred at 0°C for 20 minutes, then at room temperature for 30 minutes and finally refluxed for 5 hours. The reaction was cooled and slowly poured into a mixture of ice and 2M KOH (~100 mL). The organic solvents were evaporated off and the remaining aqueous suspension was filtered. The deep red solids obtained were dried *in vacuo* and purified by flash chromatography on silica (CH_2Cl_2/CH_3OH/Et_3N 60/1/1→60/4/1) to give **75** as reddish solids, 0.79 g (45%). ^{1}H NMR ($CDCl_3$) gave spectrum as observed in paper I.

Bis-2,3:6,7-(*N*-(1-chloroethoxycarbonyl)-iminodimethylene)-anthracene (76).
Diamine **75** (0.79 g, 1.79 mmol) was suspended in a mixture of benzene (15 mL) and 1,2-dichloroethane (15 mL) and cooled to 0°C before addition of 1-chloroethyl chloroformate (3.9 mL, 36 mmol). The reaction mixture was then refluxed for 26 hours before evaporation into the crude product **76** as a dark mass. No further purification was attempted with this material as it was used immediately in the next reaction. ^{1}H NMR ($CDCl_3$): δ 8.40 (s, 2H), 7.90 (m, 4H), 6.65 (m, 2H), 5.05-4.80 (br, 8H), 1.92 (m, 6H).

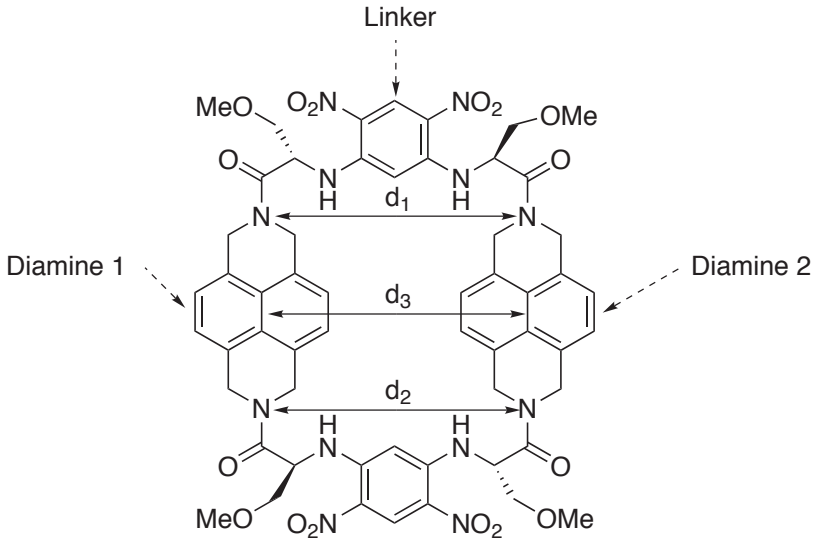
Bis-2,3:6,7-(iminodimethylene)-anthracene hydrochloride (65).
The crude material **76** was suspended in CH_3OH (60 mL) and refluxed for 20 hours. The reaction was cooled to 0°C and filtered to give the product **65** as grayish solids,

250 mg (42% from **61**). ^{1}H NMR (D_2O) gave spectra as observed in paper I though poor solubility of **65** indicated slight presence of lipophilic contaminants such as partially debenzylated **75**.

[S,S]-Bis-2,3:6,7-(*N*-[3-benzyloxy-2-(*N*-*t*-butoxycarbonylamino)-propionyl]-iminodimethylene)-anthracene (77).
Diamino dihydrochloride **65** (250 mg, 0.75 mmol) was suspended in a mixture of CH_2Cl_2 (30 mL) and THF (30 mL) together with Boc-Ser(OBn)-OH (664 mg, 2.25 mmol), EDC (860 mg, 4.5 mmol), HOBt (230 mg, 1.50 mmol), DMAP (284 mg, 3.0 mmol) and Cs_2CO_3 (1.46 g, 6.0 mmol). The reaction mixture was stirred at room temperature for 24 hours then diluted with $CHCl_3$ (100 mL) and washed with 5% HCl (2*150 mL), saturated $NaHCO_3$ (3*150 mL) and brine (3*150 mL), then dried over $MgSO_4$ and evaporated into a darkish mass. The crude product was purified with flash chromatography on silica (heptane/EtOAc 1/1→1/2) to give the product **77** as a pale mass, 26 mg (4.3%). ^{1}H NMR ($CDCl_3$): δ 8.37 (m, 2H), 7.89 (s, 2H), 7.80 (s, 2H), 7.27-7.15 (m, 10H), 5.49 (m, 2H), 5.23-4.85 (m, 10H), 4.55 (m, 4H), 3.81-3.65 (m, 4H), 1.45 (s, 18H). ^{13}C-NMR ($CDCl_3$): δ 170.11, 155.51, 137.85, 135.48, 134.90, 131.56, 131.48, 128.58, 127.92, 127.70, 126.21, 121.45, 121.32, 80.21, 73.57, 71.01, 52.01, 51.90, 51.81, 28.57. TLC (heptane/EtOAc 1/2) R_f 0.48. MS (MALDI-TOF) $[M+Na]^+$ calc 837.4, found 838.3, $[M+K]^+$ calc 853.5, found 854.5.

Macrocycle 79.
Compound **77** (26 mg, 0.032 mmol) was dissolved in $CHCl_3$ (1 mL). Triethylsilane (20 μL, 0.13 mmol) was added, followed by TFA (71 μL, 0.96 mmol). The solution was stirred at ambient temperature for 16 hours and then evaporated into a syrup. This syrup was dissolved in THF (50 mL, dried over MS) to which were added Cs_2CO_3 (62 mg, 0.19 mmol) and compound **36e** (30 mg, 0.032 mmol). The resulting suspension was refluxed for 20 hours and then evaporated. Flash chromatography (SiO_2, heptane/EtOAc 1/1→1/2→1/4) yielded **79a** as a yellow mass, 11mg (23%). ES-HRMS *m/z* calcd for $C_{84}H_{75}N_{12}O_{16}$ $[M+H]^+$ 1507.5424, found 1507.5385. TLC (heptane/EtOAc 1:4) R_f 0.41.

Summary of conformers and conformational analyses



NOC = Number Of Conformers with an energy within 5 kcal/mol relative to the lowest energy conformer **Cx**.

Conformer	diamine 1	diamine 2	linker	d_1 (Å)	d_2 (Å)	d_3 (Å)	NOC
C1	**9**	**9**	aniline	6.0	6.3	5.6	182
C2	**9**	**9**	aniline	7.4	6.8	7.5	-
C3	**9**	**9**	aniline	6.2	7.6	6.5	-
C4	**9**	**9**	urea	6.7	7.4	7.0	51
C5	**9**	**67**	urea	6.4	6.0	4.8	615
C6	**9**	**67**	aniline	4.6	6.3	6.6	276
C7	**9**	**65**	urea	6.4	6.7	6.6	215
C8	**9**	**65**	aniline	5.9	7.5	6.5	236
C9	**9**	**9**	malonic	9.8	9.5	8.8	905
C10	**9**	**9**	malonic	9.1	8.8	9.9	-
C11	**9**	**9**	acetylene	9.7	9.7	8.2	6
C12	**9**	**9**	acetylene	11.2	12.0	12.8	-

9: References

1. Steed, J.W. and Atwood, J.L.; "Supramolecular chemistry", Wiley, Chichester, **2000**.
2. Lehninger, A.L., Nelson, D.L. and Cox, M.M.;"Principles of biochemistry", Worth, New York, **1993**.
3. For some recent discussions on this topic see a: Adams, E.M., *Int J Phil Rel*, **2002**, *51*, 71-81. b: Harris, G.W., *Ethical Theo Moral Pract*, **2002**, *5*, 271-286. c: Silver, L.M., *Stud Hist Phil Biol Biomed Sci*, **2001**, *32*, 647-661.
4. McNaught, A.D. and Wilkinson, A.; "IUPAC Compendium of chemical terminology, 2nd ed", Blackwell, Oxford, **1997**.
5. Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, P.K.; "Pharmacology, 5th ed", Churchill Livingstone, Edinburgh, **2003**.
6. Kunz, H., *Angew Chem Int Ed*, **2002**, *41*, 4439-4451.
7. Noonan, G.J.; "New brewing lager beer", Brewers Publications, Boulder, **1996**.
8. Eschenmoser, A., *Angew Chem Int Ed*, **1994**, *33*, 2363.
9. Lichtenthaler, F.W., *Angew Chem Int Ed*, **1994**, *33*, 2364-2374.
10. Koshland Jr, D.E., *Angew Chem Int Ed*, **1994**, *33*, 2375-2378.
11. Davis, A.P. and Wareham, R.S., *Angew Chem Int Ed*, **1999**, *38*, 2978-2996.
12. Hartley, J.H., James, T.D. and Ward, C.J., *J Chem Soc, Perkin Trans 1*, **2000**, *3155-3184*.
13. Meyer, E.A., Castellano, R.K. and Diederich, F., *Angew Chem Int Ed*, **2003**, *42*, 1210-1250.
14. Davis, A.M. and Teague, S.J., *Angew Chem Int Ed*, **1999**, *38*, 736-749.
15. Cram, D.J. and Cram, J.M., *Acc Chem Res*, **1971**, *4*, 204-213.
16. Pascal Jr, R.A., *Eur J Org Chem*, **2004**, 3763-3771.
17. Bodwell, G.J. and Li, J., *Angew Chem Int Ed*, **2002**, *41*, 3261-3262 and references cited therein.
18. Atwood, J.L., Davies, J.E.D., MacNicol, D.D., Vögtle, F. and Lehn, J-M.; "Comprehensive supramolecular chemistry, vol 1-11", Pergamon Press, Oxford, **1996**.
19. Herm, M., Molt, O. and Schrader, T., *Chem Eur J*, **2002**, *8*, 1485-1499.
20. Molt, O., Rubeling, D., Schäfer, G. and Schrader, T., *Chem Eur J*, **2004**, *10*, 4225-4232.
21. Grawe, T., Schrader, T., Finocchiaro, P., Consiglio, G. and Failla, S., *Org Lett*, **2001**, *3*, 1597-1600.
22. Abe, H., Mawatari, Y., Teraoka, H., Fujimoto, K. and Inouye, M., *J Org Chem*, **2004**, *69*, 495-504.
23. Lara, K.O., Godoy-Alcantar, C., Rivera, I.L., Eliseev, A.V. and Yatsimirsky, A.K., *J Phys Org Chem*, **2001**, *14*, 453-462.
24. Bhattacharyya, T., Sundin, A. and Nilsson, U.J., *Tetrahedron*, **2003**, *59*, 7921-7928.
25. For some recent examples on the use of 1,4,5,8-naphthalenetetracarboxylic diimide see a: Mokhir, A., Krämer, R. and Wolf, H., *J Am Chem Soc*, **2004**, *126*, 6208-6209. b: Vignon, S.A., Jarrosson, T., Iijima, T., Tseng, H-R., Sanders, J.K.M. and Stoddart, J.F., *J Am Chem Soc*, **2004**, *126*, 9884-9885. c: Kawai, K.,

Cai, X., Sugimoto, A., Tojo, S., Fujitsuka, M. and Majima, T., *Angew Chem Int Ed*, **2004**, *43*, 2406-2409.
26. For some recent examples on the use of 2,7-diazapyrene see a: Raymo, F.M. and Cejas, M.A., *Org Lett*, **2002**, *4*, 3183-3185. b: Lilienthal, N.D., Alsafar, H., Conerty, J., Fernandez, R. Kong, C. and Smith, D.K., *Anal Chem*, **2003**, *75*, 3322-3328. c: Diehl, M.R., Steuerman, D.W., Tseng, H-R., Vignon, S.A., Star, A., Celestre, P.C., Stoddart, J.F. and Heath, J.R., *CHEMPHYSCHEM*, **2003**, *4*, 1335-1339.
27. Jazwinski, J., Blacker, A.J., Lehn, J-M., Cesario, M., Guilhem, J. and Pascard, C., *Tetrahedron Lett*, **1987**, *28*, 6057-6060.
28. Bamberger, E. and Philip, M., *Justus Liebig´s Ann Chem*, **1887**, *240*, 147-192.
29. Sotiriou-Leventis, C. and Mao, Z., *J Heterocyclic Chem*, **2000**, *37*, 1665-1667.
30. Ganin, E.V., Makarov, V.F. and Nikitin, V.I., *J Org Chem USSR*, **1987**, *23*, 1086-1089.
31. Kacprzak, K., *Synth Commun*, **2003**, *33*, 1499-1507.
32. Crockett, G.C., Swanson, B.J., Anderson, D.R. and Koch, T.H., *Synth Commun*, **1981**, *11*, 447-454.
33. Flaih, N., Pham-Huy, C. and Galons, H., *Tetrahedron Lett*, **1990**, *40*, 3697-3698.
34. Horii, Z-I., Iwata, C. and Tamura, Y., *J Org Chem*, **1961**, *26*, 2273-2276.
35. Suess, R., *Helv Chim Acta*, **1977**, *60*, 1650-1656.
36. Kamata, T. and Wasada, N., *Jpn Kokai Tokkyo Koho*, Japanese Patent 11,322,747, **1999**; *Chem Abstr*, **1999**, *131*, 337016.
37. Sotiriou-Leventis, C., Mao, Z. and Awadesh, A-M.M., *J Org Chem*, **2000**, *65*, 6017-6023.
38. Greene, T.W. and Wuts, P.G.M., "Protective groups in organic chemistry, 3rd ed", Wiley, New York, **1999**.
39. Sauer, J. and Prahl, H., *Chem Ber*, **1969**, *102*, 1917-1927.
40. Magnus, P. and Thurston, L.S., *J Org Chem*, **1991**, *56*, 1166-1170.
41. Olofson, R.A., Martz, J.T., Senet, J-P., Piteau, M. and Malfroot, T., *J Org Chem*, **1984**, *49*, 2081-2082.
42. Andricopulo, A.D., Yunes, R.A., Cechinel Filho, V., Correa, R., Filho, A.W., Santos, A.R.S. and Nunes, R.J., *Acta Farm Bonaerense*, **1998**, *17*, 219-224.
43. Brown, H.C., Murray, K.J., Murray, L.J., Snover, J.A. and Zweifel, G., *J Am Chem Soc*, **1960**, *82*, 4233-4241.
44. Han. S-Y. and Kim, Y-A., *Tetrahedron*, **2004**, *60*, 2447-2467.
45. Armarego, W.L.F. and Perrin, D.D.,"Purification of laboratory chemicals, 4th ed", Butterworth-Heinemann, Oxford, **1996**.
46. Moroder, L. in "Houben-Weyl, 4th ed", Vol E22a; Goodman, M., Felix, A., Moroder, L. and Toniolo, C. Eds; Thieme, Stuttgart, **2002**, 28.
47. Mehta, A., Jaouhari, R., Benson, T.J. and Douglas, K.T., *Tetrahedron Lett*, **1992**, *33*, 5441-5444.
48. Golds, E.E. and Braun, P.E., *J Biol Chem*, **1978**, *253*, 8162-8170.
49. Marfey, P., *Carlsberg Res Commun*, **1984**, *49*, 591-596.
50. Adamson, J.G., Hoang, T., Crivici, A. and Lajoie, G.A., *Anal Biochem*, **1992**, *202*, 210-214.
51. Shivanyuk, A., Far, A.R. and Rebek Jr, J., *Org Lett*, **2002**, *4*, 1555-1558.
52. Arnusch, C.J. and Pieters R.J., *Tetrahedron Lett*, **2004**, *45*, 4153-4156.
53. Halgren, T.A., *J Comput Chem*, **1999**, *20*, 720-729.
54. Bunnet, J.F. and Zahler, R.E., *Chem Rev*, **1951**, *49*, 273-412.
55. Miller, J.F., "Aromatic Nucleophilic Substitution", Elsevier, Amsterdam, **1968**.

56. Chambers, R.D., Seabury, M.J. and Williams, D.L.H., *J Chem Soc, Perkin Trans 1*, **1988**, 255-257.
57. Chambers, R.D., Close, D., Musgrave, W.K.R., Waterhouse, J.S. and Williams, D.L.H., *J Chem Soc, Perkin Trans 1*, **1977**, 1774-1778.
58. Vlasov, V.M., *Russ Chem Rev*, **2003**, *72*, 681-703.
59. Persson, J. and Matsson, O., *J Org Chem*, **1998**, *63*, 9348-9350.
60. Bernasconi, C.F., de Rossi, R.H. and Schmid, P., *J Am Chem Soc*, **1977**, *99*, 4090-4101.
61. Persson, J., Axelsson, S. and Matsson, O., *J Am Chem Soc*, **1996**, *118*, 20-23.
62. Kumar, A., *Molec Phys*, **1967**, *12*, 593-596.
63. Ruggli, P., *Justus Liebig´s Ann Chem*, **1917**, *412*, 1-13.
64. Luttringshaus, A. and Ziegler, K., *Justus Liebig´s Ann Chem*, **1937**, *528*, 155-161.
65. Lindley, J., *Tetrahedron*, **1984**, *40*, 1433-1456.
66. Ma, D., Zhang, Y., Yao, J., Wu, S. and Tao, F., *J Am Chem Soc*, **1998**, *120*, 12459-12467.
67. Hore, P.J.; "Nuclear Magnetic Resonance", Oxford University Press, Oxford, **1995**.
68. Rossi, S., Kyne, G.M., Turner, D.L., Wells, N.J. and Kilburn, J.D., *Angew Chem Int Ed,* **2002**, *41*, 4233-4236.
69. Gowda, D.C., *Indian J Chem*, **2002**, *41B*, 1064-1067.
70. Lott, R.S., Chauhan, V.S. and Stammer, C.H., *J Chem Soc, Chem Comm,* **1979**, 495-496.
71. Kumar, S., Singh, H. and Sharma, R., *J Indian Chem Soc*, **2003**, *80*, 1111-1128.
72. Sasaki, S-I., MIzuno, M., Naemura, K. and Yoshito, T., *J Org Chem*, **2000**, *65*, 275-283.
73. Bordwell, F.G., Algrim, D.J. and Harrelson Jr, J.A., *J Am Chem Soc*, **1988**, *110*, 5903-5904.
74. Nowick, J.S., Holmes, D.L., Noronha, G., Smith, E.H., Nguyen, T.M. and Huang, S-L., *J Org Chem*, **1996**, *61*, 3929-3934.
75. Gastaldi, S., Weinreb, S.M. and Stien, D., *J Org Chem*, **2000**, *65*, 3239-3240.
76. Bigi, F., Maggi, R. and Sartori, G., *Green Chemistry*, **2000**, *2*, 140-148.
77. Freer, R. and McKillop, A., *Synth Comm*, **1996**, *26*, 331-349.
78. Thavonekham, B., *SYNTHESIS*, **1997**, 1189-1194.
79. Castro, E.A., Ruiz, M.G. and Santos, J.G., *Int J Chem Kinetics*, **2001**, *33*, 281-287.
80. Menger, F.M. and Glass, L.E., *J Org Chem*, **1974**, *39*, 2469-2470.
81. Koh, H.J., Kim, O.S., Lee, H.W. and Lee, I., *J Phys Org Chem*, **1997**, 725-730.
82. Konakahara, T., Ozaki, T., Sato, K. and Gold, B., *SYNTHESIS*, **1993**, 103-106.
83. Hubener, G., Göhring, W., Musiol, H-J., Moroder, L. and Wunsch, E., *Chem Pept Protein*, **1993**, *5*, 73-79.
84. Hubener, G., Göhring, W., Musiol, H-J., and Moroder, L., *Pept Res*, **1992**, *5*, 287-292.
85. Bodanszky, M. and Martinez, J., *SYNTHESIS*, **1981**, 333-356.
86. Tam, J.P., Riemen, M.W. and Merrifield, R.B., *Pept Res*, **1988**, *1*, 6-18.
87. Tam, J.P., Wong, T-W., Riemen, M.W., Tjoeng, F-S. and Merrifield, R.B., *Tetrahedron Lett*, **1979**, *20*, 4033-4036.
88. Karlström, A. and Unden, A., *Int J Peptide Protein Res*, **1996**, *48*, 305-311.
89. Grundberg, H.E., Wendt, O.F. and Nilsson, U.J., *Tetrahedron Lett*, **2004**, *45*, 6083-6085.

90. Grundberg, H.; "Synthesis and binding studies of 9,10-disubstituted anthracene and dibenzobarrelene-based receptors for recognition of carbohydrates in water", Doctoral thesis, Lund University, **2004**.
91. Connors, K.A.; "Binding constants", Wiley, New York, **1987**.
92. Escuder, B. Rowan, A.E., Feiters, M.C. and Nolte, R.J.M., *Tetrahedron*, **2004**, *60*, 291-300.