



# LUND UNIVERSITY

## Macrocyclic Carbohydrate/Amino Acid Hybrid Molecules - Synthesis and Evaluation as Artificial Receptors

Billing, Johan

2005

[Link to publication](#)

*Citation for published version (APA):*

Billing, J. (2005). *Macrocyclic Carbohydrate/Amino Acid Hybrid Molecules - Synthesis and Evaluation as Artificial Receptors*. [Doctoral Thesis (compilation), Centre for Analysis and Synthesis]. Organic 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

# Macrocyclic Carbohydrate/Amino Acid Hybrid Molecules

Synthesis and Evaluation as Artificial Receptors

**Johan Billing**

---

Organic Chemistry  
Lund, 2005



**LUND**  
UNIVERSITY

Akademisk avhandling som för avläggandet av teknisk doktorexamen vid tekniska fakulteten vid Lunds Universitet kommer att offentligen försvaras på Kemicentrum, sal K:C, fredagen den 11 mars 2005, 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 summarizes the accompanying papers. These have either already been published or are manuscripts at various stages (*in press, submitted or in manuscript*).

© Johan Billing  
Organic Chemistry  
Center for Chemistry and Chemical Engineering  
Lund University  
P.O. Box 124  
SE-221 00 LUND  
SWEDEN

ISBN 91-628-6413-0

Printed by Media-Tryck, Lund University, Lund.

Organization LUND UNIVERSITY  Organic Chemistry PO Box 124 SE-221 00 Lund SWEDEN	Document name DOCTORAL DISSERTATION	
	Date of issue 2005-01-27	
	Sponsoring organization Swedish Research Council, Swedish Strategic Research Foundation and the program "Glycoconjugates in Biological Systems" sponsored by the Swedish Strategic Research Foundation.	
Author(s) Johan Billing		
Title and subtitle Macrocytic Carbohydrate/Amino Acid Hybrid Molecules - Synthesis and Evaluation as Artificial Receptors		
Abstract  Methods were developed for the synthesis of three different types of macrocyclic carbohydrate/amino acid hybrid molecules. In the synthesis of the first type of macrocycles, a glucosamine derivative was oxidized at C6 and the obtained sugar amino acid was coupled to tripeptides. The resulting hybrids were transformed into dimers and cyclized in a macrolactamization step to obtain C <sub>2</sub> -symmetric macrocycles. In the second type of macrocycles, the same sugar amino acid was used together with tyrosine to prepare a C <sub>3</sub> -symmetric macrocycle with alternating sugar amino acid and tyrosine residues. In the synthesis of the third type of macrocycles, Cu(I)-catalysed 1,3-dipolar cycloaddition of azides and acetylenes was employed to form C <sub>2</sub> -symmetric carbohydrate/amino acid hybrid molecules containing two 1,2,3-triazole units.  The macrocycles that were water-soluble were screened for affinity towards biomolecules. Weak, but significant, affinity was found for dAMP, dGMP, serotonin and caffeine for some of the macrocycles (K <sub>a</sub> ≈ 10 M <sup>-1</sup> ) which indicates that molecules of this type have potential as artificial receptors. Possibilities to extend the developed methods to prepare further types of macrocycles are discussed.		
Key words: Cyclic peptides, macrocycles, sugar amino acids, artificial receptors, molecular recognition, 1,2,3-triazoles, 1,3-dipolar cycloaddition		
Classification system and/or index terms (if any):		
Supplementary bibliographical information:		Language English
ISSN and key title:		ISBN 91-628-6413-0
Recipient's notes	Number of pages 86	Price
	Security classification	

Distribution by (name and address)

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 Johan Billing

Date 2005-01-27



# Populärvetenskaplig sammanfattning

---

Proteiner är en typ av molekyler som är inblandade i så gott som alla processer i levande organismer. Naturen använder proteiner bland annat för att omvandla näringsämnen till energi i ämnesomsättningen, för att transportera molekyler in och ut genom cellmembran och för att binda ihop celler så att vävnad kan bildas. I de flesta av sina uppgifter måste proteiner kunna känna igen och binda till molekyler till sin omgivning. I vissa fall, som vid vävnadsbildning, är bindningen själva huvudfunktionen hos proteinerna, och i många andra fall så regleras proteinernas funktion genom interaktioner med hormoner och andra signalsubstanser. Vissa proteiner (enzymer) kan även hjälpa till att omvandla bundna molekyler i kemiska reaktioner.

Proteiner binder till andra molekyler i bindningsfickor på ytan, och forskning kring konstgjorda receptorer går ut på att tillverka molekyler som kan härma struktur och funktion hos dessa bindningsfickor. Proteiner är oftast mycket stora och komplexa molekyler, så detta kan vara till nytta för att med hjälp av enklare modellsystem kunna studera hur inbindning egentligen går till. Det finns även andra tillämpningar av konstgjorda receptorer, t ex kan receptorer som ändrar färg eller någon annan mätbar egenskap när de kommer i kontakt med en molekyl man vill detektera användas som kemiska sensorer. Tänkbara tillämpningar finns även inom läkemedelsområdet, men det är dock fortfarande osäkert om det går att göra effektiva och säkra läkemedel baserade på konstgjorda receptorer.

Konstgjorda receptorer är generellt sett utformade antingen som ringar eller pincetter. I forskningen som presenteras i den här avhandlingen har metoder utvecklats för att tillverka ringar som innehåller kolhydrater (socker) och aminosyror. Receptorerna binder till vissa biologiskt viktiga molekyler som dAMP och dGMP (beståndsdelar i arvsmassan), serotonin (en signalsubstans i hjärnan) och koffein (en uppiggande naturprodukt), men bara med låg bindningsstyrka. Detta visar att ringar bestående av kolhydrater och aminosyror kan fungera som konstgjorda receptorer även om stark inbindning ännu inte har uppnåtts med denna typ av molekyler.



# List of Papers

---

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

- I. Johan F. Billing and Ulf J. Nilsson  
Cyclic peptides containing a  $\delta$ -sugar amino acid–synthesis and evaluation as artificial receptors.  
*Tetrahedron* **2005**, *61*, 863-874.
- II. Johan F. Billing and Ulf J. Nilsson  
Synthesis of a  $C_3$ -symmetric macrocycle with alternating sugar amino acid and tyrosine residues.  
*Tetrahedron Letters* **2005**, *46*, 991-993.
- III. Johan F. Billing and Ulf J. Nilsson  
 $C_2$ -symmetric macrocyclic carbohydrate/amino acid hybrids through copper(I)-catalyzed formation of 1,2,3-triazoles.  
*In manuscript*.
- IV. Johan F. Billing and Ulf J. Nilsson  
Synthesis of an *O*-benzyl protected  $\delta$ -sugar amino acid *tert*-butyl ester.  
*In manuscript*.





# Abbreviations

---

AA	amino acid
Ac	acetyl
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
<i>t</i> -Bu	<i>tert</i> -butyl
Bz	benzoyl
CBz	benzyloxycarbonyl
CPK	Corey-Pauling-Koltun
dA	2'-deoxyadenosine
dAMP	2'-deoxyadenosine 5'-monophosphate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
dCMP	2'-deoxycytidine 5'-monophosphate
DEAD	diethyl azodicarboxylate
dGMP	2'-deoxyguanosine 5'-monophosphate
DIC	<i>N,N'</i> -diisopropylcarbodiimide
DIPEA	<i>N,N</i> -diisopropylethylamine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulphoxide
DMTMM	4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride
DOPA	3,4-dihydroxyphenylalanine
DPPA	diphenylphosphoryl azide
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
Et	ethyl
Fmoc	(9-fluorenyl)methoxycarbonyl
FRET	fluorescence resonance energy transfer
HAPyU	1-(1-pyrrolidinyl-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i> ]pyridin-1-yl-methylene)pyrrolidinium hexafluorophosphate <i>N</i> -oxide
HATU	1-[bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i> ]pyridinium hexafluorophosphate 3-oxide
<i>O</i> -HATU	<i>N</i> -[(dimethylamino)(1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i> ]pyridin-1-yloxy)-methylene]- <i>N</i> -methylmethanaminium hexafluorophosphate
HBTU	1-[bis(dimethylamino)methylene]-1 <i>H</i> -benzotriazolium hexafluorophosphate 3-oxide
<i>O</i> -HBTU	<i>N</i> -[(1 <i>H</i> -benzotriazol-1-yloxy)(dimethylamino)methylene]- <i>N</i> -methylmethanaminium hexafluorophosphate
HMPA	hexamethylphosphoramide
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
IIDQ	2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline
LG	leaving group
MALDI-TOF	matrix-assisted laser desorption/ionization–time of flight
Me	methyl
MMFFs	Merck molecular force field (static)
Mtr	4-methoxy-2,3,6-trimethylbenzenesulphonyl

NMR	nuclear magnetic resonance
PG	protective group
Phth	phthaloyl
PNP	<i>para</i> -nitrophenyl
PyAOP	(7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyBrOP	bromotripyrrolidinophosphonium hexafluorophosphate
SAA	sugar amino acid
Su	<i>N</i> -succinimidyl
TBTU	1-[bis(dimethylamino)methylene]-1 <i>H</i> -benzotriazolium tetrafluoroborate 3-oxide
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy, free radical
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMP	thymidine 5'-monophosphate
Ts	<i>para</i> -toluenesulphonyl

Three-letter symbols for amino acids and monosaccharides are used following the IUPAC/IUBMB recommendations (*Pure Appl. Chem.* **1984**, 56, 595-624 and *Pure Appl. Chem.* **1996**, 68, 1919-2008).

# Contents

---

<b>1 INTRODUCTION.....</b>	<b>1</b>
1.1 ARTIFICIAL RECEPTORS .....	1
1.2 DESIGN OF ARTIFICIAL RECEPTORS .....	3
1.3 OBJECTIVES OF THE RESEARCH .....	4
<b>2 CARBOHYDRATES AND AMINO ACIDS AS BUILDING BLOCKS FOR ARTIFICIAL RECEPTORS .....</b>	<b>5</b>
2.1 RECEPTORS CONTAINING CARBOHYDRATES.....	5
2.2 RECEPTORS CONTAINING AMINO ACIDS AND PEPTIDES .....	7
<b>3 SUGAR AMINO ACIDS .....</b>	<b>11</b>
3.1 INTRODUCTION TO SUGAR AMINO ACIDS.....	11
3.2 USES OF SUGAR AMINO ACIDS .....	12
3.2.1 Scaffolds for combinatorial chemistry .....	12
3.2.2 Carbohydrate analogues.....	12
3.2.3 Peptide mimetics .....	13
3.2.4 Artificial receptors.....	14
<b>4 PRINCIPLES OF PEPTIDE CHEMISTRY .....</b>	<b>15</b>
4.1 THE FORMATION OF PEPTIDE BONDS.....	15
4.2 PROTECTIVE GROUPS .....	17
4.3 EPIMERIZATION .....	18
<b>5 C<sub>2</sub>-SYMMETRIC CYCLIC PEPTIDES CONTAINING A δ-SUGAR AMINO ACID .....</b>	<b>21</b>
5.1 DESIGN CONSIDERATIONS .....	21
5.2 SYNTHESIS .....	22
5.2.1 Synthesis of the Fmoc protected sugar amino acid .....	22
5.2.2 Synthesis of tripeptide <i>tert</i> -butyl esters .....	23
5.2.3 Synthesis of the macrocycles.....	24
5.3 EVALUATION AS ARTIFICIAL RECEPTORS.....	28
5.4 COMPUTATIONAL STUDIES .....	34
5.5 CONCLUSIONS.....	36
<b>6 C<sub>3</sub>-SYMMETRIC CYCLIC PEPTIDES WITH ALTERNATING TYROSINE AND SUGAR AMINO ACID RESIDUES .....</b>	<b>37</b>
6.1 DESIGN CONSIDERATIONS .....	37
6.2 SYNTHESIS .....	38
6.3 COMPUTATIONAL STUDIES .....	40
6.4 OUTLINE OF AN IMPROVED SYNTHESIS .....	41
6.4.1 Protective groups that can be cleaved by hydrogenolysis.....	41
6.4.2 Reducing the risk of epimerization.....	42
6.5 CONCLUSIONS.....	43
<b>7 1,2,3-TRIAZOLE-CONTAINING MACROCYCLES WITH C<sub>2</sub>-SYMMETRY .....</b>	<b>45</b>
7.1 DESIGN CONSIDERATIONS .....	45
7.2 SYNTHESIS .....	46
7.2.1 Synthesis of the azidoaminosugar.....	46

7.2.2 Synthesis of the <i>N</i> -propiolyl dipeptides.....	47
7.2.3 Synthesis of the macrocycles.....	49
7.3 COMPUTATIONAL STUDIES.....	52
7.4 BINDING STUDIES.....	53
7.5 CONCLUSIONS.....	55
<b>8 SUMMARY AND FUTURE PERSPECTIVES.....</b>	<b>57</b>
<b>9 ACKNOWLEDGEMENTS.....</b>	<b>61</b>
<b>10 SUPPLEMENTARY DATA.....</b>	<b>63</b>
10.1 PREPARATION OF HAPYU.....	63
10.2 SYNTHESIS OF REFERENCE TRIPEPTIDE AC-TYR-ARG-TYR-OME.....	63
10.3 SYNTHESIS OF <b>35</b> .....	65
10.4 SYNTHESIS OF <i>N</i> -PROPIOLYL-L-TYROSINE <i>TERT</i> -BUTYL ESTER <b>40</b> .....	66
<b>11 REFERENCES.....</b>	<b>67</b>

# 1

## Introduction

---

### 1.1 Artificial receptors

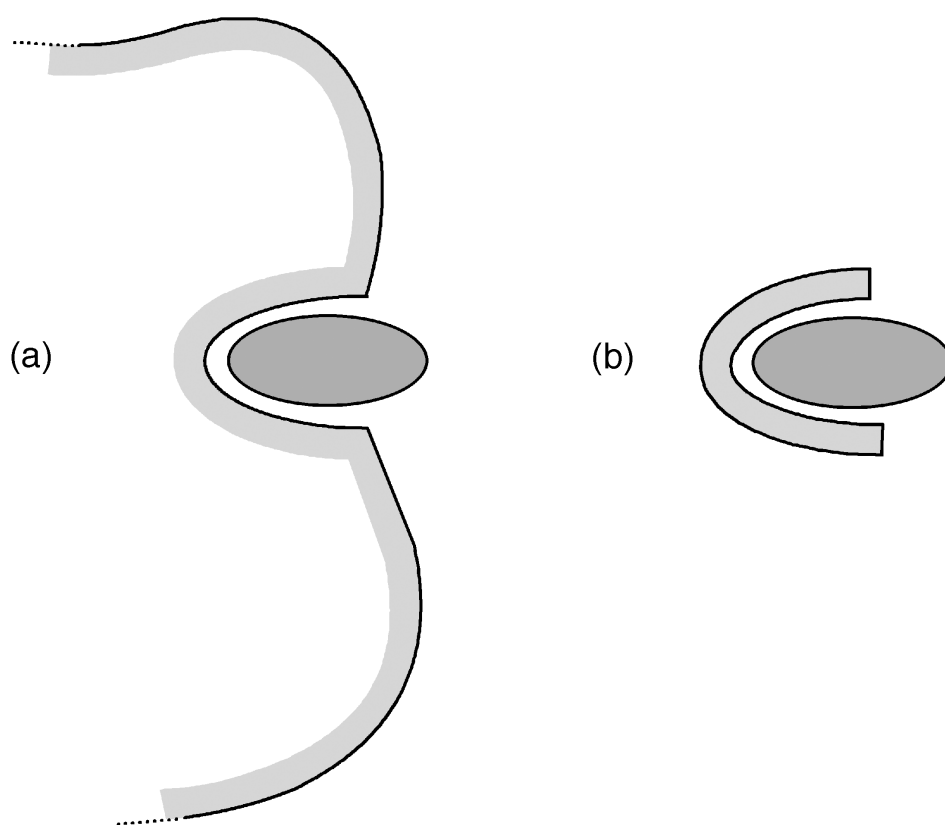
Proteins are versatile molecules that are involved in almost all aspects of life.<sup>1</sup> Proteins convert nutrients to energy in the metabolism, transport molecules through cell membranes, and mediate cell adhesion in tissue formation. Proteins also have many other functions and it would be difficult to find a process in a living cell where proteins are not involved. In many of these processes, proteins need to bind to other molecules in binding sites on their surfaces. In some cases, such as cell adhesion molecules,<sup>2</sup> binding is the main function of the protein, and in many other cases, the functions of proteins are regulated through interactions with hormones and other molecules. Enzymes go one step further and have catalytic sites where reactions of bound molecules are catalysed.

Since proteins are so important in biological systems, it is not surprising that there is a strong interest in obtaining deeper and more detailed understanding of the mechanisms and driving forces behind protein binding events. The synthesis of artificial receptors<sup>3</sup> is one tool among many in this undertaking. Artificial receptors are organic molecules that can bind to other molecules, referred to as ligands, in a fashion similar to binding sites in proteins\* (Figure 1.1). Since artificial receptors are generally much smaller molecules than proteins, they can be used as more simple model systems to investigate the thermodynamic driving forces and structural requirements involved in a binding event in greater detail.<sup>4</sup>

Furthermore, artificial receptors are not only interesting as tools for studying molecular recognition, but may also be useful in their own right in analytical chemistry. Cyclodextrins are cyclic oligosaccharides with the ability to bind to many smaller molecules, and this property has been utilized in chromatographic systems. Cyclodextrins can either be linked to the stationary phase or added to the mobile phase to improve

---

\* In biological terminology, the word *receptor* implies a physiological effect upon ligand binding, and some researchers thus avoid the term *artificial receptor* and prefer to use the more general term *host* instead.



**Figure 1.1** Schematic pictures of (a) a protein binding a ligand in a binding site on the surface, and (b) an artificial receptor binding a ligand.

separation, and their chiral nature can also make it possible to differentiate between enantiomers.<sup>5</sup> In principle, artificial receptors can be utilized in the same way, and most progress so far appears to have been made with calixarenes.<sup>6</sup> Artificial receptors where an easily observable property, such as absorbance or fluorescence, changes upon binding can be utilized as chemosensors<sup>7-10</sup> and be used analogously to pH indicators to detect and quantify analytes. Recently, sensing systems that rely on the displacement of an indicator from a receptor have been developed. These systems have been used for the detection of phosphate<sup>11</sup> and pyrophosphate<sup>12</sup> ions in water and for the determination of malate and tartrate in wines, grape juice and grape must,<sup>13-14</sup> citrate in beverages<sup>15</sup> and the time of aging of whisky.<sup>16</sup> It has been suggested that fluorescent sensors for biomolecules could become very important tools in molecular biology,<sup>17</sup> and many other analytical applications can be envisioned provided that the appropriate sensors can be developed.

The first steps towards therapeutic applications of artificial receptors have also been made.<sup>18</sup> Vancomycin is a natural antibiotic that inhibits cell wall growth by binding to the C-terminal sequence of a mucopeptide precursor. However, bacteria are

increasingly developing resistance to this antibiotic by modifying the C-terminal and new antibiotics need to be found. Artificial receptors that can bind to the modified sequence may have potential as new antibiotics, and some research towards this has already been made.<sup>19,20</sup> In another example of research towards therapeutic applications of artificial receptors, a receptor has been developed that blocks the farnesylation of the protein Ras, a process known to be involved in some types of cancer.<sup>21</sup> It has also been shown that cyclophanes<sup>22</sup> and modified cyclodextrins<sup>23</sup> can be used to reverse the effects of some skeletal muscle relaxants. The reversal drugs that are used today when only a temporary effect is sought cause many side effects, and research on artificial receptors may lead to better alternatives in the future. However, the viability of artificial receptors as drugs is still uncertain at this stage.

## 1.2 Design of artificial receptors

Proteins bind other molecules through a combination of electrostatic interactions, such as ion pairing and hydrogen bonding, and hydrophobic effects, i.e. the association of hydrophobic regions in a polar solvent.<sup>24</sup> These non-covalent interactions are generally not very strong, but by using multiple interactions, protein binding sites can often bind the ligands with both high affinity and high specificity.

With the exception of boronate esters,<sup>25</sup> artificial receptors use the same forces for binding their ligands.<sup>26</sup> Most of the early work on artificial receptors was done in organic solvents, and those receptors often relied heavily on hydrogen bonding to bind their ligands.<sup>3</sup> However, it is more interesting to construct biomimetic artificial receptors that can bind ligands in water, and hydrogen bonds and other electrostatic forces are much weaker in water than in organic solvents due to the higher polarity of the solvent. Furthermore, water is both a hydrogen bond donor and acceptor and the hydrogen bonds between receptor and ligand need to be stronger than hydrogen bonds between receptor and solvent in order to create a net force for binding. On the other hand, hydrophobic interactions and other interactions with aromatic rings can be very important in aqueous solution.<sup>27</sup> Artificial receptors for use in aqueous solution are often amphiphilic molecules with both aromatic regions and regions rich in hydrogen-bonding sites and ionic groups, thus allowing both association between aromatic regions in receptor and ligand and polar interactions.

The structural elements of artificial receptors are generally arranged in a macrocyclic ring or as a tweezer-like structure to form a binding site that is complementary to the ligand. The advantage of constructing a macrocycle is that it leads



to a more rigid molecule where the structural elements can be preorganized<sup>28</sup> in a good geometry for binding, thus reducing the amount of free energy that needs to be spent on conformational changes in the binding event. Still, the receptor should be flexible enough to allow for an induced fit,<sup>29</sup> and typically a compromise between rigidity and flexibility needs to be reached.

### **1.3 Objectives of the research**

The aim of the research presented in this thesis was to explore the use of macrocyclic carbohydrate/amino acid hybrid molecules as artificial receptors for biomolecules in aqueous solution. The macrocycles were to be prepared mainly using the methods of peptide chemistry with the carbohydrates in the form of sugar amino acids. It was expected that interesting artificial receptors or leads towards artificial receptors would be found, and it was also expected that the research would expand the body of knowledge concerning the synthesis of such macrocycles.

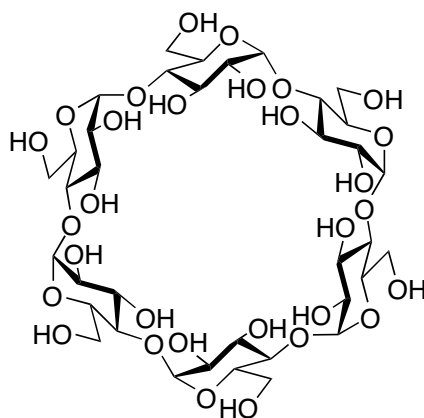
# 2

## Carbohydrates and Amino Acids as Building Blocks for Artificial Receptors

---

### 2.1 Receptors containing carbohydrates

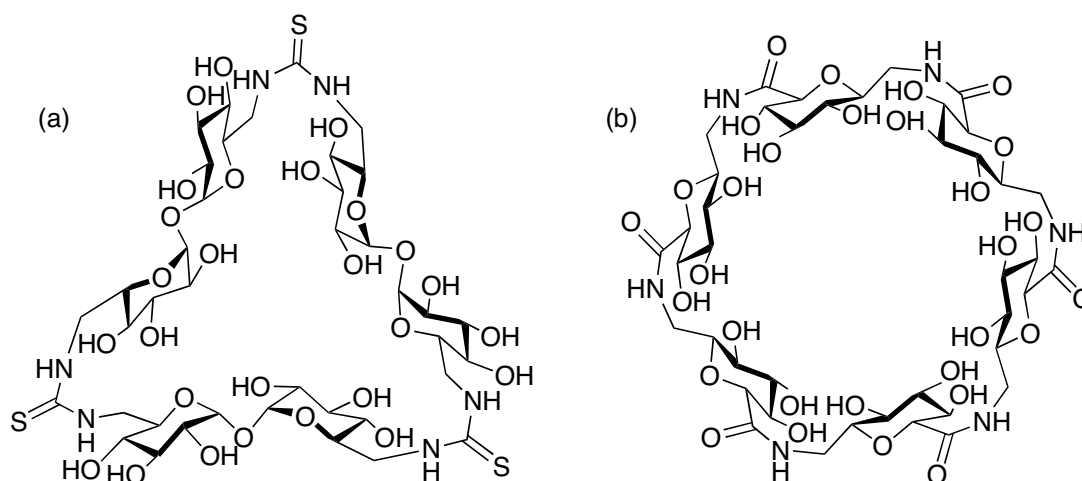
The naturally occurring cyclodextrins (Figure 2.1) were among the first receptor molecules to be studied.<sup>3a</sup> Cyclodextrins are cyclic oligosaccharides that are now known to form complexes with many organic and inorganic compounds,<sup>30</sup> and the chemical literature contains a wealth of information about the binding properties of both natural and modified cyclodextrins.<sup>31</sup> Carbohydrates have also been used in many other ways in artificial receptors and this section aims to give examples of those attempts with the focus on artificial receptors in aqueous solution.



**Figure 2.1**  $\alpha$ -Cyclodextrin. Larger cyclodextrins with seven ( $\beta$ -cyclodextrin), eight ( $\gamma$ -cyclodextrin) or even more glucose units are also known.

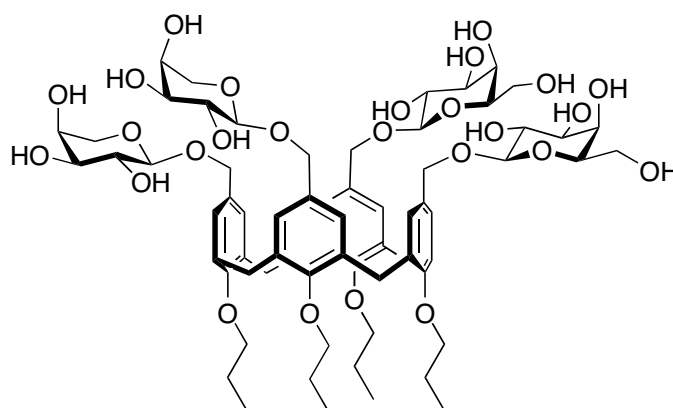
A number of cyclodextrin analogues have been prepared, such as the cyclotrehalin prepared by Mellet, García Fernández and co-workers<sup>32</sup> (Figure 2.2a). This molecule can be considered to be a reversed cyclodextrin since the other face of the glucose units is

oriented towards the central cavity. The cyclotrehalin was found to bind weakly to benzoate anions in water ( $K_a=8 \text{ M}^{-1}$ ) and stronger to 2-naphthalene sulphonate anions ( $K_a=235 \text{ M}^{-1}$ ) and 1-adamantanecarboxylate anions ( $K_a=46\,000 \text{ M}^{-1}$ ). Another example of a cyclodextrin analogue is the cyclohexamer of a sugar amino acid prepared by Kessler and co-workers<sup>33</sup> (Figure 2.2b). The cyclohexamer was found to bind to *p*-nitrophenol and benzoic acid in water, but no binding constants were reported.



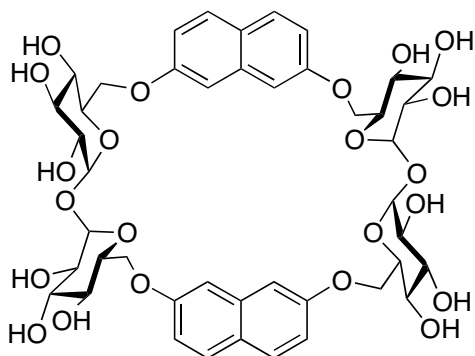
**Figure 2.2** Cyclodextrin analogues. (a) Cyclotrehalin by Mellet, García Fernández and co-workers.<sup>32</sup> (b) Cyclic hexamer of a sugar amino acid by Kessler and co-workers.<sup>33</sup>

Carbohydrates have also been used together with other building blocks. Dondoni and co-workers attached carbohydrates to a calix[4]arene to give calixsugars, and one of the resulting molecules (Figure 2.3) was found to bind D-glucosamine hydrochloride in MeOH- $d_4$  or DMSO- $d_6$  in preliminary studies, but unfortunately no details were given.<sup>34</sup> Binding studies in water could not be performed due to solubility problems.



**Figure 2.3** Calixsugar by Dondoni and co-workers.<sup>34</sup>

In a more successful attempt to use carbohydrates in artificial receptors, Penadés and co-workers incorporated carbohydrates into a cyclophane to produce a glycochane (Figure 2.4) that could bind *p*-nitrophenyl glycosides in water with binding constants up to 242 M<sup>-1</sup> and 2,4-dinitrophenyl glycosides with binding constants up to 1200 M<sup>-1</sup>.<sup>35</sup>

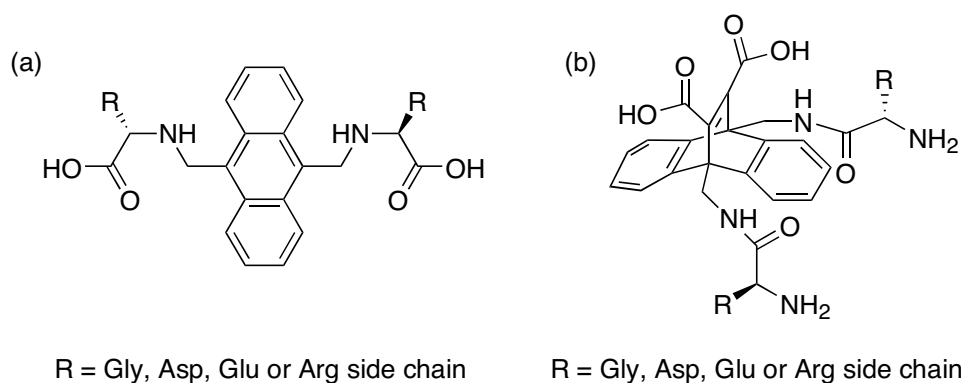


**Figure 2.4** Glycochane by Penadés and co-workers.<sup>35</sup>

## 2.2 Receptors containing amino acids and peptides

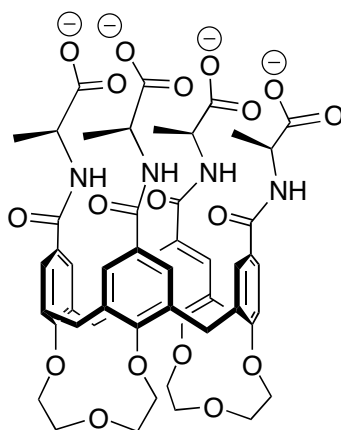
Amino acids have frequently been used in artificial receptors. They are attractive building blocks since the structure of the receptors can be easily varied by changing the amino acid(s) and since the available amino acids have a large diversity in chemical properties. Combinatorial methods can be used, and efficient tweezer-like artificial receptors have been obtained in this way by coupling variable peptide chains to an invariant core.<sup>36</sup> Examples of the use of peptides and amino acids in artificial receptors will be given in this section with an emphasis on artificial receptors in aqueous solution.

In the simplest cases of amino acid-containing receptors, amino acids have been coupled to aromatic building blocks to create amphiphilic molecules that can act as artificial receptors. In our laboratory, this has been done with derivatives of anthracene<sup>37</sup> and dibenzobarrelene<sup>38</sup> that were combined with glycine, aspartic acid, glutamic acid or arginine (Figure 2.5). The anthracene-based receptor with arginine was found to bind neutral *p*-nitrophenyl glycosides with  $K_a \approx 100 \text{ M}^{-1}$  and negatively charged *p*-nitrophenyl glucuronide and *p*-nitrophenyl galacturonide with  $K_a \approx 200\text{-}300 \text{ M}^{-1}$  in water. The dibenzobarrelene-based receptor with arginine was found to bind *p*-nitrophenyl glucuronide with  $K_a = 50\text{-}67 \text{ M}^{-1}$  and *p*-nitrophenyl galacturonide with  $K_a = 77\text{-}111 \text{ M}^{-1}$  in water.



**Figure 2.5** Simple receptors based on (a) anthracene,<sup>37</sup> and (b) dibenzobarrelene.<sup>38</sup>

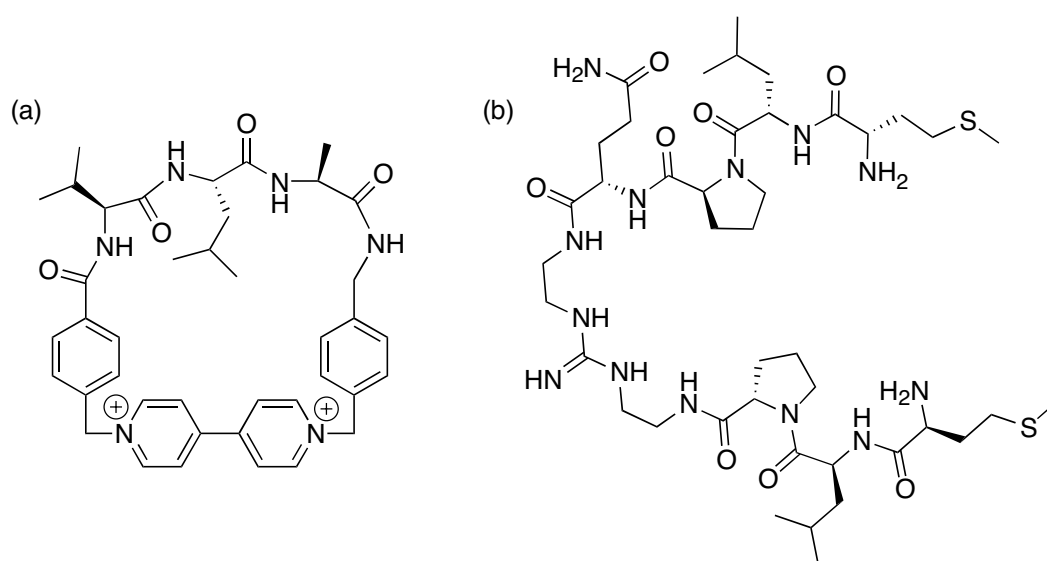
There are many examples where amino acids or short peptides have been coupled to calixarenes to obtain artificial receptors.<sup>39</sup> Many of those receptors were only studied in organic solvents, but there are also examples in water, such as the receptor prepared by Ungaro and co-workers<sup>40</sup> (Figure 2.6). In that receptor, four alanine units were coupled to a calix[4]arene that had been rigidified with two di(ethylene glycol) bridges at the lower rim, and the resulting molecule was found to bind to aromatic ammonium salts, amino acids and amino acid methyl esters. The more hydrophobic molecules were bound most strongly with binding constants up to  $710 \text{ M}^{-1}$  for L-tryptophan methyl ester and  $1440 \text{ M}^{-1}$  for trimethylanilinium chloride.



**Figure 2.6** Peptidocalix[4]arene by Ungaro and co-workers.<sup>40</sup>

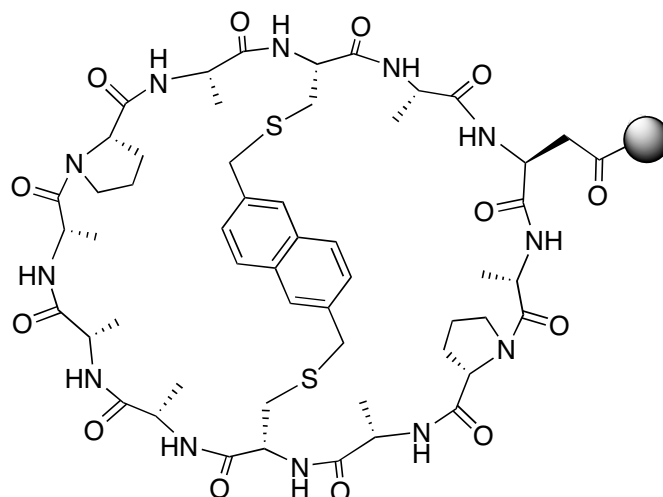
Entire peptide sequences have also been incorporated in artificial receptors. Mallouk and co-workers incorporated a tripeptide (Val-Leu-Ala) into a cyclophane and obtained a receptor that could bind to some pharmaceutically interesting molecules in a

mixture of water and acetone<sup>41</sup> (Figure 2.7a). The binding constants were small ( $K_a \leq 39 \text{ M}^{-1}$ ), but an interesting feature of the receptor was that it bound considerably stronger to (*R*)-DOPA than to (*S*)-DOPA. Kilburn and co-workers developed a receptor that could bind to a dye-labelled Glu(*O**t*Bu)-Ser(*t*Bu)-Val peptide.<sup>42</sup> The receptor had a guanidino group that could interact with the terminal carboxylate group of the peptide and two side-chains that could interact with the rest of the peptide chain (Figure 2.7b). The receptor bound the peptide with  $K_a = 8 \times 10^4 \text{ M}^{-1}$  while the enantiomeric peptide was only bound with  $K_a = 8 \times 10^3 \text{ M}^{-1}$ . Sugimoto, Miyoshi and Zou have also reported that some pentapeptides can bind very strongly to D-erythrose and D-galactose.<sup>43</sup>



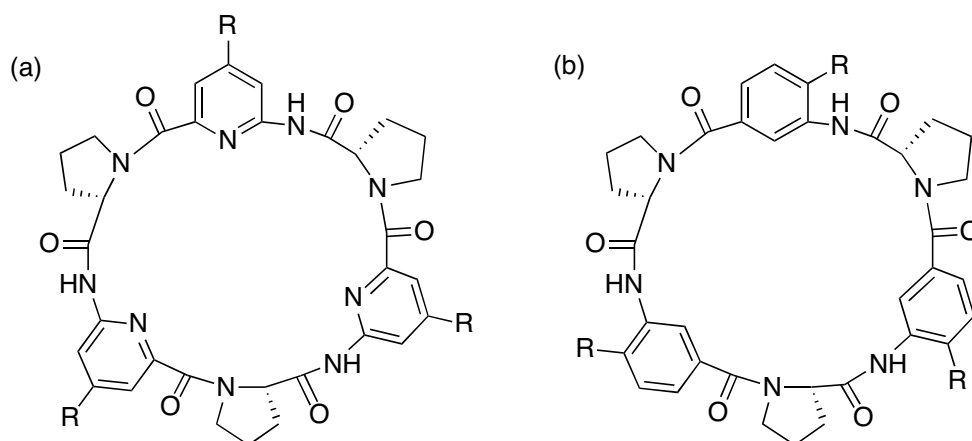
**Figure 2.7** Examples of receptors that contain peptide sequences. (a) Cyclophane by Mallouk and co-workers.<sup>41</sup> (b) Peptide receptor by Kilburn and co-workers.<sup>42</sup>

Benito and Meldal have investigated the use of cyclic peptides as receptors.<sup>44</sup> A cysteine-containing dodecapeptide was synthesized on solid phase and was bridged with different aromatic chains. The binding to nitrotyrosine-labelled cellobiose, maltose and lactose was studied with fluorescence resonance energy transfer (FRET) with the receptors still linked to the solid phase, and cellobiose was found to bind more strongly than maltose and lactose in acetonitrile:water 3:1 when a naphthalene bridge was used (Figure 2.8).



**Figure 2.8** Naphthalene-bridged cyclic dodecapeptide by Benito and Meldal.<sup>44</sup>

Kubik and co-workers have also studied the use of cyclic peptide-like molecules as artificial receptors. It was found that a macrocycle with alternating residues of proline and 6-aminopicolinic acid could bind anions in aqueous solution (Figure 2.9a).<sup>45</sup> If an excess of receptor was used, a 2:1 complex would be formed and the binding strength could be enhanced by linking two macrocycles together.<sup>46,47</sup> It has also been shown that this macrocycle can bind octyl and methyl glycosides when carboxylate-containing groups are present on the aromatic rings, but only in chloroform containing small amounts of methanol.<sup>48</sup> Similar macrocycles (Figure 2.9b) have been shown to bind cations in organic solvents.<sup>49</sup>



**Figure 2.9** General structures of receptors by Kubik and co-workers.

# 3

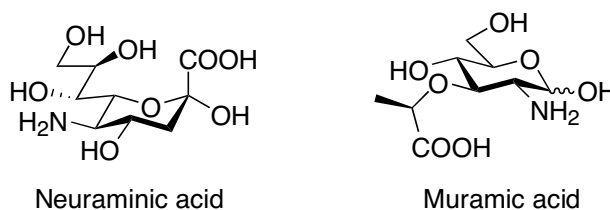
## Sugar Amino Acids

---

### 3.1 Introduction to sugar amino acids

Sugar amino acids<sup>50</sup> (also known as glycosamino acids) are carbohydrates that contain at least one amino group and one carboxylic acid group, either attached directly to the carbohydrate or via a short spacer. Sugar amino acids are useful building blocks in organic chemistry and can easily be coupled to amines and carboxylic acids using methods developed for peptide chemistry.

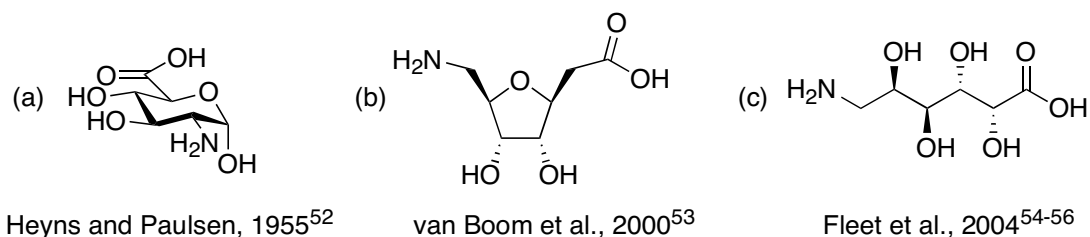
A number of natural sugar amino acids are known with derivatives of neuraminic acid and muramic acid (Figure 3.1) as important examples. Sugar amino acids have also been found as components in natural antibiotics<sup>51</sup> and several other examples of natural sugar amino acids are also known.



**Figure 3.1** Examples of naturally occurring sugar amino acids.

The first synthetic sugar amino acid was prepared by Heyns and Paulsen in 1955 by selective oxidation of C6 of a glucosamine derivative.<sup>52</sup> A wide range of sugar amino acids have been synthesized since then and many pyranoid, furanoid and open-chain sugar amino acids can be found in the literature (Figure 3.2).



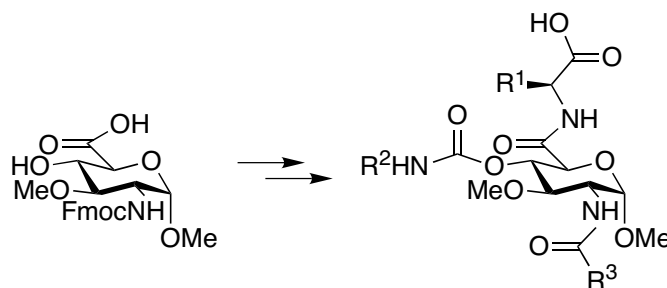


**Figure 3.2** Examples of (a) pyranoid, (b) furanoid, and (c) open-chain sugar amino acids (shown here as the corresponding fully deprotected structures).

## 3.2 Uses of sugar amino acids

### 3.2.1 Scaffolds for combinatorial chemistry

Pyranoid and furanoid sugar amino acids contain three different kinds of functionality (hydroxyl groups, amino groups and carboxylic acid) in a defined geometry and so are good candidates for molecular scaffolds in combinatorial chemistry. An illustrative example is the 384 member library prepared by Sofia and co-workers<sup>57</sup> (Figure 3.3).

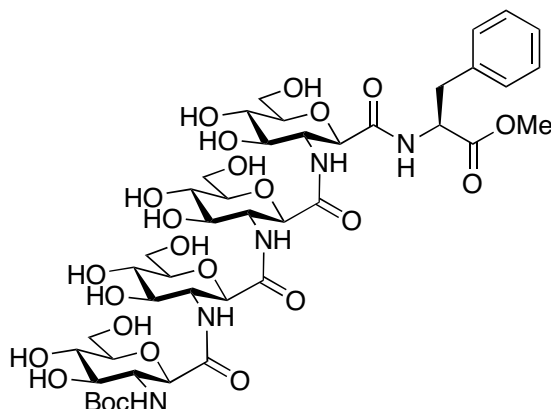


**Figure 3.3** Use of a sugar amino acid as a scaffold for combinatorial chemistry.<sup>57</sup>

### 3.2.2 Carbohydrate analogues

It was first proposed by Fuchs and Lehmann in 1975<sup>58</sup> and later by Nicolaou and co-workers in 1995<sup>59</sup> that sugar amino acids could be linked together to form interesting carbohydrate analogues, and Nicolaou termed such molecules carbopeptoids. The first fully characterized carbopeptoid was prepared by Ichikawa and co-workers<sup>60</sup> (Figure 3.4) and was found to inhibit HIV infection in CD4 cells when *O*-sulphated. Many other linear oligomers of sugar amino acids have now also been prepared and several of them have been found to form interesting secondary structures. An analogue of a branched oligosaccharide has been synthesized, but was found to be biologically inactive.<sup>61</sup> A

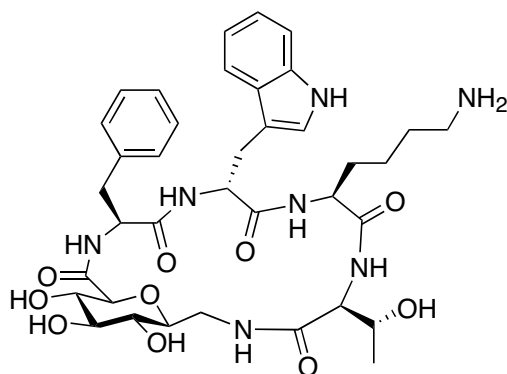
number of cyclic oligomers of sugar amino acids have also been prepared, such as the cyclodextrin analogue by Kessler and co-workers<sup>33</sup> (see Section 2.1).



**Figure 3.4** First synthetic carbopeptoid.<sup>60</sup>

### 3.2.3 Peptide mimetics

Sugar amino acids have lately seen much use in peptide mimetics. A sugar amino acid typically has the same size as a dipeptide, but is more rigid and can be incorporated into cyclic peptides to control their conformation. The first example of this is the biologically active analogue of the cyclic peptide somatostatin that was prepared by Graf von Roedern and Kessler<sup>62,63</sup> (Figure 3.5). In this peptide the sugar amino acid replaced the Phe-Pro sequence and conformational analysis showed that the sugar amino acid mimicked a  $\beta$  turn. Sugar amino acids have also been incorporated into other cyclic peptides to prepare Arg-Gly-Asp-containing integrin inhibitors<sup>64,65</sup> and further analogues of somatostatin.<sup>66</sup>



**Figure 3.5** Somatostatin analogue containing a sugar amino acid.<sup>62,63</sup>

### **3.2.4 Artificial receptors**

It has been proposed that cyclic sugar amino acid/amino acid hybrids may have potential as artificial receptors,<sup>53,67</sup> but little work has been done in this area and the only known artificial receptor containing a sugar amino acid is the cyclodextrin analogue discussed in Section 2.1. Two cyclic sugar amino acid/amino acid hybrids have been evaluated as artificial receptors in the work presented in this thesis, but the use of sugar amino acids in artificial receptors still remains little explored.

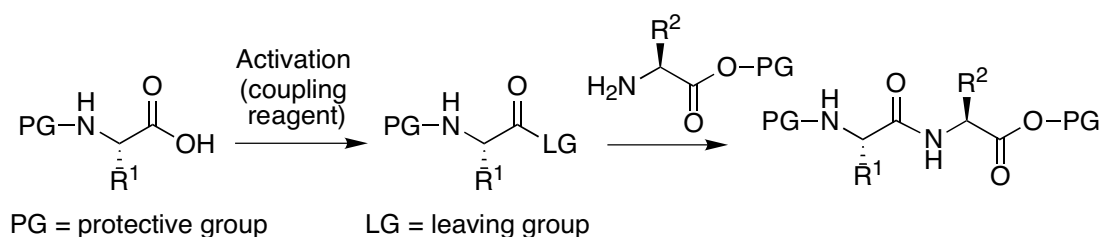
# 4

## Principles of Peptide Chemistry

---

### 4.1 The formation of peptide bonds

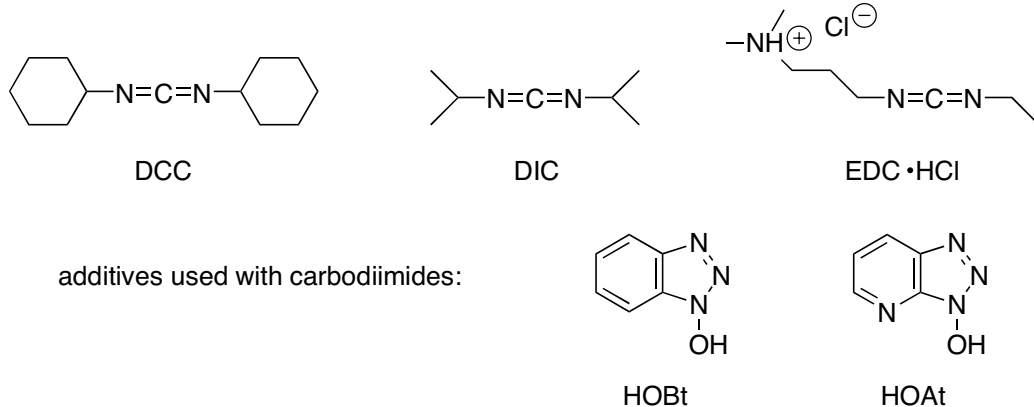
Amino acids in peptides and proteins are linked through amide bonds (often simply referred to as peptide bonds) and the formation of such bonds is the most central reaction in peptide chemistry.<sup>68</sup> This reaction is often called a peptide coupling and reagents for such a reaction are known as coupling reagents. The role of the coupling reagent is to introduce a leaving group into the carboxylic acid of one amino acid so that it can react with the amine of another amino acid to form an amide bond between them (Figure 4.1). The introduction of a leaving group is often referred to as activation of the amino acid.



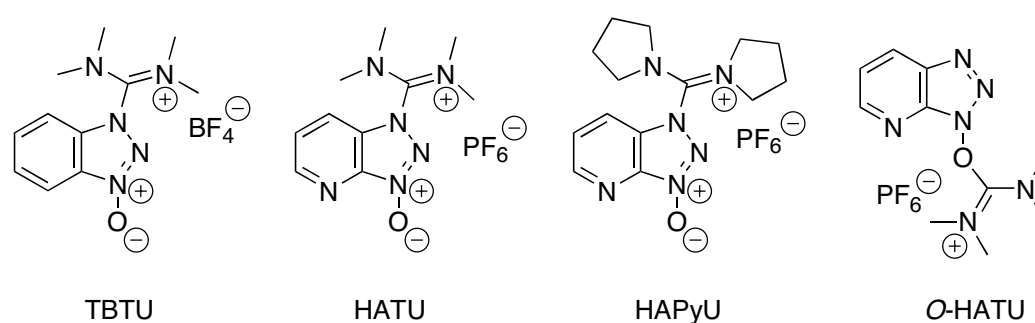
**Figure 4.1** A peptide coupling reaction

During the 100 years that have passed since the birth of peptide chemistry, a plethora of coupling reagents have been developed.<sup>69</sup> The three most common types of coupling reagents today are the carbodiimides, the guanidinium/uronium salts and the phosphonium salts. Reagents that create mixed anhydrides between an amino acid and a monoester of carbonic acid also remain popular and useful. Examples of these types of coupling reagents are shown in Figure 4.2 and their characteristics are discussed below:

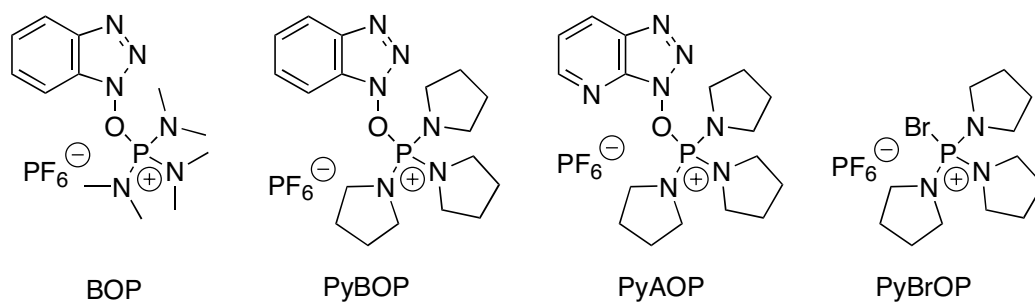
### Carbodiimides



### Guanidinium/Uronium salts



### Phosphonium salts



### Reagents for preparing mixed anhydrides

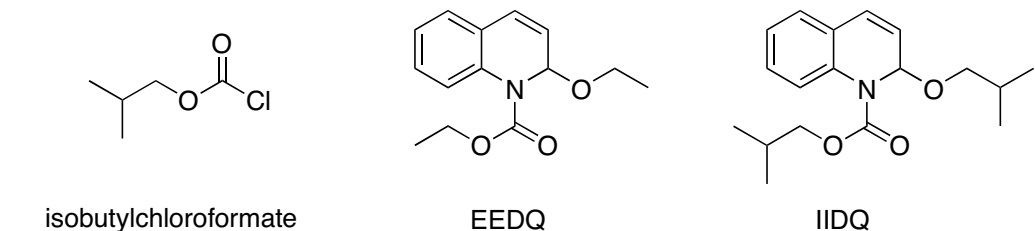


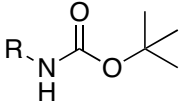
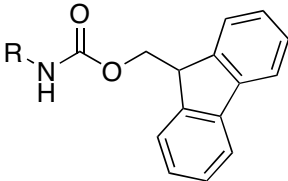
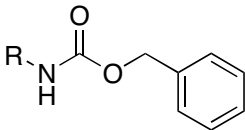
Figure 4.2 Examples of common coupling reagents

- Carbodiimides have been used for a long time and DCC was the first coupling reagent of this class to be developed.<sup>70</sup> The main disadvantage of the carbodiimides is that the urea that is formed in the coupling reaction can be difficult to remove in the purification. The *N,N'*-dicyclohexylurea formed from DCC precipitates from most solvents and can often be filtered off, although small amounts may remain. DIC gives the more soluble *N,N'*-diisopropylurea which can be advantageous in some situations, e.g. solid phase peptide synthesis. EDC yields a very polar urea that can easily be removed from the product through liquid-liquid extraction or chromatography. Carbodiimides are almost always used together with additives such as HOBT<sup>71</sup> or HOAt<sup>72</sup> to suppress racemization and to improve yields.
- Guanidinium salts are efficient coupling reagents.<sup>73</sup> Originally, these reagents were believed to be uronium salts, but the HBTU and HATU reagents have now been shown to be guanidinium salts<sup>74</sup> and that is likely also the case for the other members of this class. Some confusion remains as the abbreviations HATU, TBTU etc are still used even though they indicate that the compounds are uronium salts. The true uronium salts of HBTU and HATU, *O*-HBTU and *O*-HATU, have also been prepared and have been shown to be more reactive than their guanidinium counterparts.<sup>74</sup>
- Phosphonium salts are also commonly used in peptide synthesis, although they have not been used in the work presented in this thesis. BOP<sup>75</sup> is prepared from the carcinogenic substance HMPA which is released again in the coupling reaction, and PyBOP has been developed as a more benign alternative.<sup>76</sup> PyBrOP has been shown to be a valuable reagent for coupling hindered secondary amines.<sup>77,78</sup>
- Mixed anhydrides are most often prepared by treating the amino acid with a chloroformate such as isobutylchloroformate in the presence of a base. Another way to introduce a mixed anhydride is to use EEDQ<sup>79</sup> or IIDQ<sup>80</sup> which in contrast to chloroformates can be used in the presence of the amine.

## 4.2 Protective groups

Another important aspect of peptide chemistry is the choice of protective groups. As can be inferred from Figure 4.1, the amino acids need to be suitably protected or they will polymerize in the course of the reaction. The most common protective groups for the amino group are carbamate protective groups such as Boc, Fmoc and CBz (Table 4.1).

**Table 4.1** Common carbamate protective groups.

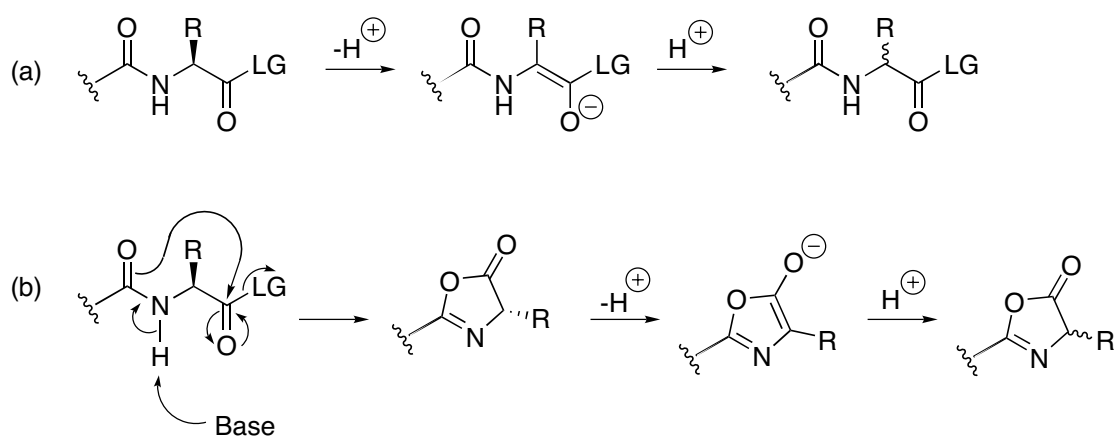
Abbreviation	Name	Structure	Cleavage conditions
Boc	<i>tert</i> -butoxycarbonyl		TFA or other acid
Fmoc	(9-fluorenyl)-methoxycarbonyl		piperidine or other base
CBz or Z	benzyloxycarbonyl		hydrogenolysis

In solid-phase peptide synthesis, the carboxylic acid functionality is generally protected by being the point of attachment to the solid phase. In solution, esters such as methyl, allyl, benzyl or *tert*-butyl can be used.

The amino acid side chains also need to be protected in many cases and a large number of protective groups have been developed. The choice of side-chain protective groups depends heavily on the choice of amino protective group as it is important that the amino protective group can be cleaved without affecting the side chain.

### 4.3 Epimerization

The most troublesome side reaction in peptide chemistry is epimerization of the activated amino acid. Epimerization can occur in two ways, either through direct proton abstraction or via oxazol-5(4*H*)-one formation (Figure 4.3).<sup>81,82</sup> The activation of an amino acid makes it more prone to epimerization through direct abstraction since the leaving group generally is electron-withdrawing and so makes the  $\alpha$ -hydrogen more acidic. However, epimerization through direct abstraction is normally not a major problem in peptide chemistry. Instead, it is epimerization via oxazolones that is the concern. Once the oxazolone has been formed, it epimerizes rapidly via an aromatic anion. Since oxazol-5(4*H*)-ones react with amines to give amides, this will not necessarily lead to a loss of yield, only to partially epimerized product.



**Figure 4.3** Epimerization mechanisms in peptide couplings. (a) Direct abstraction. (b) Oxazol-5(4*H*)-one formation.

Fortunately, carbamate-protected amino acids are much less prone to epimerization than peptides, and if only one carbamate-protected amino acid is added to the growing peptide chain at a time, epimerization will most often occur only to a negligible extent. This is the reason why peptide synthesis is generally performed in the C→N direction.

However, in some cases it is desirable to couple two peptide segments together to form a longer peptide, a reaction known as a segment condensation. In those cases it is necessary to choose conditions where epimerization is minimized. Since the formation of oxazolone is base-catalysed, one way to avoid epimerization is to use a weaker base or, even better, to use a coupling reagent that can be used without a base, such as the carbodiimides.<sup>83</sup> Lowering the temperature can also help decrease epimerization, and HOAt-based reagents are often superior to HOBt-based reagents. Studies have also shown that there generally is less epimerization in relatively non-polar solvents such as  $\text{CH}_2\text{Cl}_2$  and THF than in very polar solvents such as DMF and  $\text{CH}_3\text{CN}$ .<sup>84</sup> It has also been shown that certain metal cations can suppress epimerization<sup>85,86</sup> and  $\text{CuCl}_2$  has been found to be particularly effective.



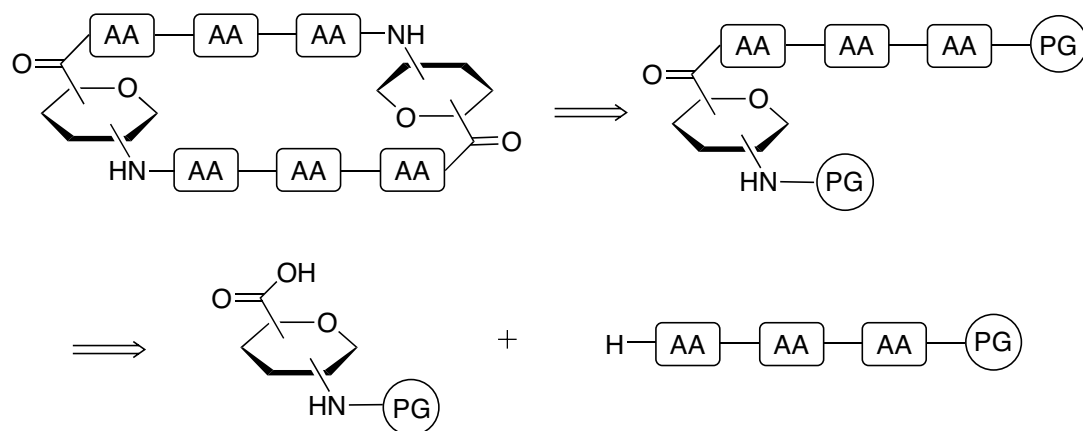


# 5

## $C_2$ -Symmetric Cyclic Peptides Containing a $\delta$ -Sugar Amino Acid

### 5.1 Design considerations

The goal of the project was to combine amino acids and carbohydrates to produce macrocyclic molecules that would be able to act as artificial receptors. The carbohydrates were to be in the form of sugar amino acids so that the macrocycles could be prepared using peptide chemistry. It was also decided that the macrocycles were to be  $C_2$ -symmetric, allowing them to be prepared through dimerization of smaller precursors. It was envisioned that ligands could be bound in the centre of the molecules, and CPK models were constructed to examine how large the macrocycles would need to be in order to form a binding site large enough for small ligands such as monosaccharides. It was judged that two sugar amino acids and six  $\alpha$ -amino acids would be suitable, and so the general structure shown in Figure 5.1 was chosen for the synthetic targets (Paper I).



**Figure 5.1** General structure and retrosynthetic analysis of the synthetic targets.

The  $\delta$ -sugar amino acid obtained by oxidation of the C6 in glucosamine was chosen to be used as the sugar amino acid since it was expected that a sugar amino acid with diametrically opposed amino and carboxylic acid groups would introduce rigidity into the macrocyclic ring and prevent it from collapsing. Since the macrocycles should be amphiphilic molecules that can use both hydrogen bonding and hydrophobic effects to bind ligands, aromatic amino acids were to be used. Of the four available natural aromatic amino acids phenylalanine, tyrosine, histidine and tryptophan, tyrosine was chosen because the side-chain hydroxyl group was expected to help increase the water-solubility. Macrocycles with glutamic acid or arginine as the central amino acid residue in the tripeptide chain were also to be prepared so that the effect of a positive or negative charge on the binding properties could be examined.

## 5.2 Synthesis

In order to be able to synthesize the macrocycles in a stepwise cyclodimerization, it was necessary to choose orthogonal amino and carboxylic acid protective groups, and the Fmoc amino protective group together with *tert*-butyl esters was deemed to be a suitable combination. Thus, an Fmoc-protected sugar amino acid and tripeptide *tert*-butyl esters needed to be prepared.

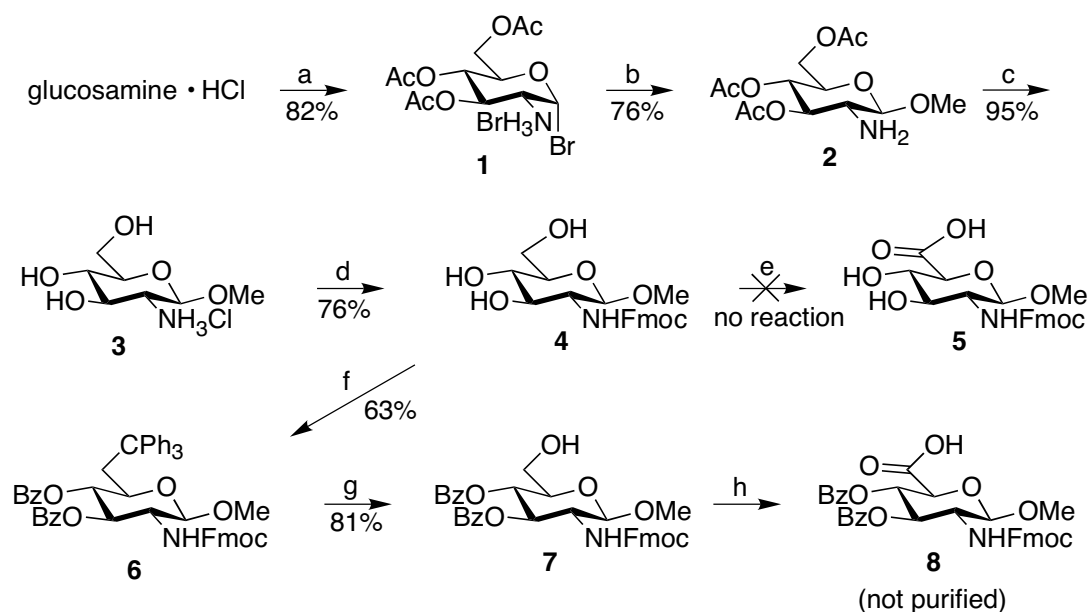
### 5.2.1 Synthesis of the Fmoc protected sugar amino acid

The synthesis started from glucosamine hydrochloride which was transformed into the known tri-*O*-acetylated methyl pyranoside **2** in a two-step procedure using a combination of previously described methods<sup>87,88</sup> (Scheme 5.1). Attempts to deacetylate **2** using base-catalysed transesterification with Me<sub>2</sub>NEt in MeOH gave approx. 12% of an *N*-acetyl side-product, while acidic transesterification cleanly produced known hydrochloride **3**.<sup>89</sup> The Fmoc group was introduced selectively with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu) to give **4**. At this point, attempts were made to selectively oxidize C6 of the Fmoc-protected carbohydrate derivative **4** to obtain sugar amino acid **5**. The reaction was attempted with TEMPO in a two-phase CH<sub>2</sub>Cl<sub>2</sub>/water system<sup>90</sup> and also in EtOAc/water, water, and acetonitrile/water, but at most only traces of product could be seen on TLC and detected with MALDI-TOF mass spectrometry.\* Instead, the primary hydroxyl group of **4** was protected as the triphenylmethyl ether and the secondary hydroxyl groups as benzoates to give **6**.<sup>†</sup> The

---

\*The reason for the sluggish reaction may have been the poor solubility of the amphiphilic starting material. The reaction has recently been accomplished in a 1:1 mixture of THF and water in 81% yield.<sup>91</sup>

† Acetates were initially used, but were found to be too prone to migration in later Fmoc cleavage steps.

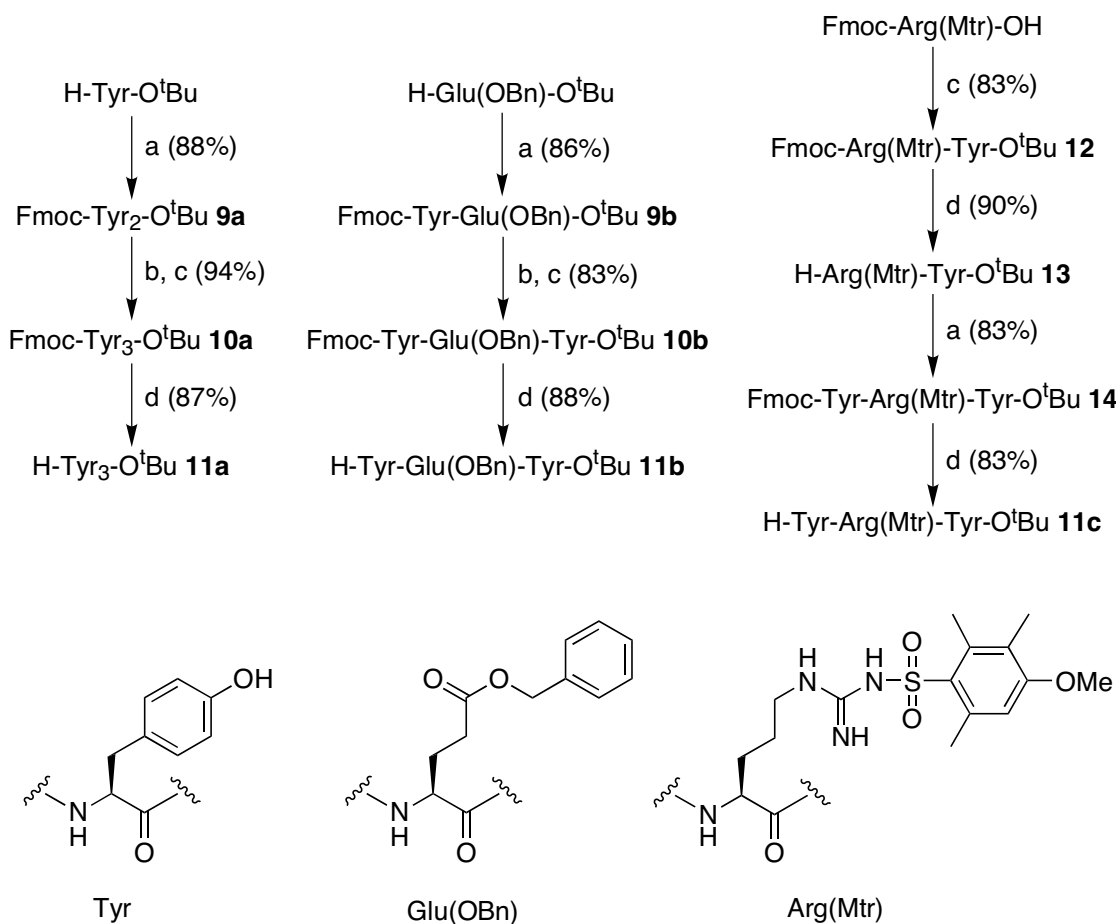


**Scheme 5.1** Synthesis of sugar amino acid **8**. Reaction conditions: (a) AcBr (neat), 3 days; (b) MeOH, pyridine, 1 h; (c) HCl/MeOH, 24 h; (d) Fmoc-OSu, NaHCO<sub>3</sub>, 15 h; (e) TEMPO, KBr, Bu<sub>4</sub>NCl, NaClO, NaHCO<sub>3</sub>(aq), CH<sub>2</sub>Cl<sub>2</sub>, no reaction; (f) Chlorotriphenylmethane, pyridine, 85°C, 2 h, then BzCl, pyridine, r.t., 4 h; (g) HBr/AcOH, 3 min; (h) CrO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>(aq), acetone, 1.5 h.

triphenylmethyl ether was cleaved with hydrogen bromide in acetic acid to give **7** which could be cleanly oxidized with Jones' reagent to give the Fmoc-protected sugar amino acid **8**. The purification of the Fmoc-protected sugar amino acid was difficult, and it was judged to be better to directly use the crude product later in the synthesis of the macrocycles. A similar sequence leading to the  $\alpha$  anomer of the same sugar amino acid has been disclosed by others.<sup>92</sup>

### 5.2.2 Synthesis of tripeptide *tert*-butyl esters

Three different tripeptide *tert*-butyl esters were prepared for inclusion into macrocycles; H-Tyr-Tyr-Tyr-O<sup>t</sup>Bu **11a**, H-Tyr-Glu(OBn)-Tyr-O<sup>t</sup>Bu **11b** and H-Tyr-Arg(Mtr)-Tyr-O<sup>t</sup>Bu **11c** (Scheme 5.2). Peptide couplings were performed with EDC•HCl, HOBT and *N*-methylmorpholine in THF, *tert*-butyl esters were cleaved with 33% TFA in CH<sub>2</sub>Cl<sub>2</sub> with Et<sub>3</sub>SiH as a scavenger,<sup>93</sup> and piperidine was used to cleave the Fmoc group. All three peptides could be prepared in good yields without protection of the tyrosine side chain. The side chain of glutamic acid was protected as the benzyl ester and the guanidino group in the arginine side chain was protected with the Mtr group.

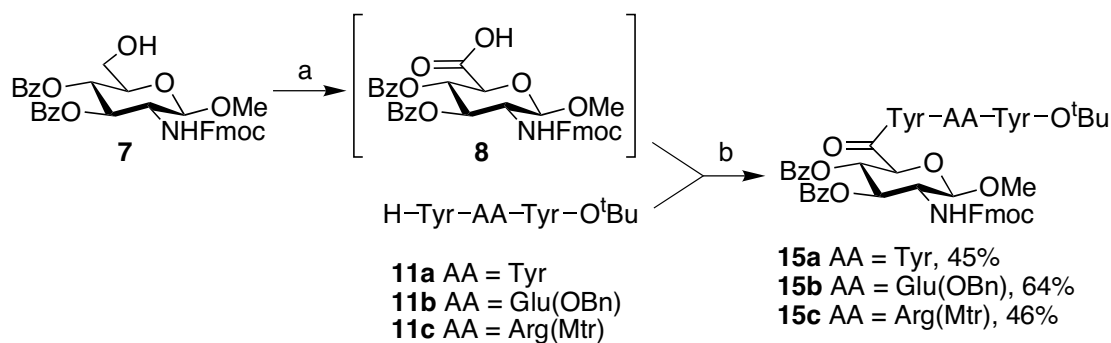


**Scheme 5.2** Synthesis of tripeptide *tert*-butyl esters **11a-c**. Reaction conditions: (a) Fmoc-Tyr-OH, EDC•HCl, HOBT, *N*-methylmorpholine, THF, 16 h; (b) TFA, Et<sub>3</sub>SiH, CH<sub>2</sub>Cl<sub>2</sub>, 4 h; (c) H-Tyr-O<sup>t</sup>Bu, EDC•HCl, HOBT, *N*-methylmorpholine, THF, 16 h; (d) piperidine, CH<sub>2</sub>Cl<sub>2</sub>, 30 min.

### 5.2.3 Synthesis of the macrocycles

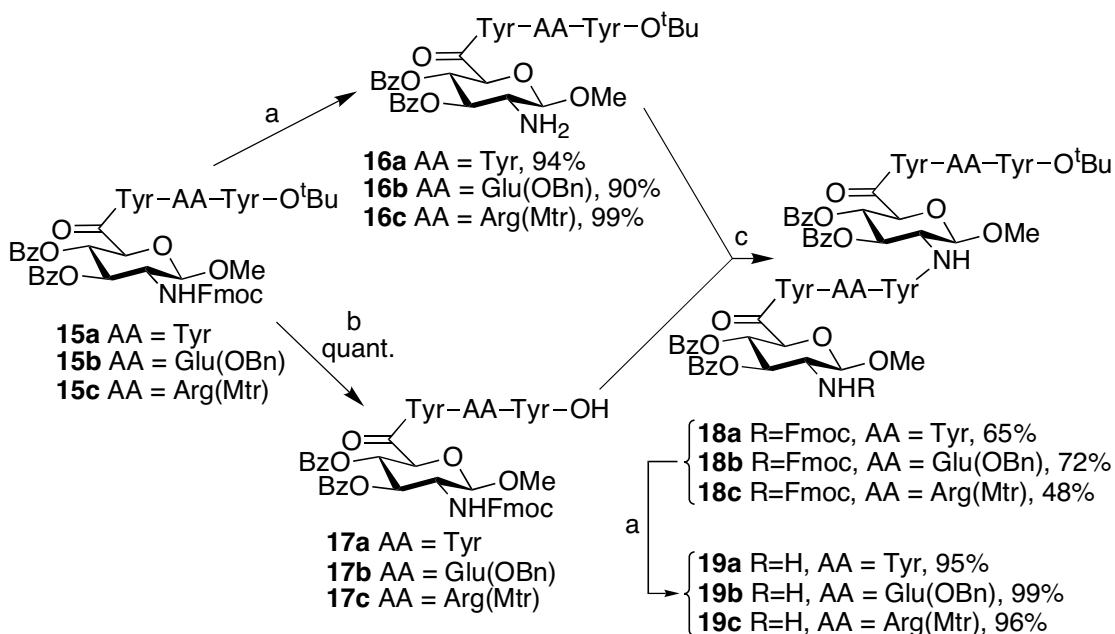
Sugar amino acid precursor **7** was oxidized with Jones' reagent and directly coupled to a tripeptide *tert*-butyl ester **11a-c** to give sugar amino acid/amino acid hybrids **15a-c** (Scheme 5.3). The orthogonally protected hybrids **15a-c** could be either deprotected at the N-terminal using DBU in the presence of a solid-phase thiol as a scavenger for the liberated dibenzofulvene<sup>94</sup> to give **16a-c** or at the C-terminal using TFA and Et<sub>3</sub>SiH in CH<sub>2</sub>Cl<sub>2</sub><sup>93</sup> to give **17a-c** (Scheme 5.4). The Mtr group has previously been reported to be stable at 25% TFA in CH<sub>2</sub>Cl<sub>2</sub>,<sup>65</sup> and this was also the case during the *tert*-butyl ester cleavage at 33% TFA in CH<sub>2</sub>Cl<sub>2</sub>.

The N-deprotected hybrids **16a-c** and the C-deprotected hybrids **17a-c** were now ready to be coupled together in a segment condensation. In the first attempt,



**Scheme 5.3** Synthesis of sugar amino acid/amino acid hybrids **15a-c**. Reaction conditions: (a) CrO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>(aq), acetone, 1.5 h; (b) EDC•HCl, HOBT, *N*-methylmorpholine, THF, 16 h.

EDC•HCl/HOBT/*N*-methylmorpholine was used, but the results were unsatisfactory as there was a high degree of epimerization (approx. 20% epimer of **18a** formed according to <sup>1</sup>H NMR). Instead, a carbodiimide-mediated base-free coupling method using DIC and HOBT was used to couple **16a-c** and **17a-c** to get **18a-c** without any visible epimerization in the <sup>1</sup>H NMR spectrum. These conditions are known to work well in difficult segment condensations.<sup>83</sup> The linear dimers were then *N*-deprotected with the solid-phase scavenger as above to give **19a-c**.



**Scheme 5.4** Synthesis of the linear dimers **19a-c**. Reaction conditions: (a) DBU, *N*-(2-mercaptoethyl)aminomethyl polystyrene, 6 h; (b) TFA, Et<sub>3</sub>SiH, CH<sub>2</sub>Cl<sub>2</sub>, 3-4 h; (c) DIC, HOBT, THF, 16 h;

It was now time to cyclize the linear dimers. To evaluate cyclization methods, a portion of **19a** was C-protected and cyclization attempts were made using the following conditions:

- EDC•HCl/HOBt and *N*-methylmorpholine in THF.
- DIC/HOBt, both with and without DIPEA in both THF and DMF.
- DPPA, both with NaHCO<sub>3</sub> in DMF and with DIPEA in THF.
- TBTU, HOBt and DIPEA in THF.
- HATU, both with DIPEA and 2,4,6-collidine in THF.
- HAPyU, both with DIPEA and 2,4,6-collidine in THF.

All attempts were carried out at 1 mM concentration of the linear starting material. The reaction mixtures were analysed with MALDI-TOF mass spectrometry and only TBTU, HATU, and HAPyU gave the macrocycle as a major product.

The HATU and HAPyU reagents have been reported to give less epimerization than TBTU in the cyclization of pentapeptides.<sup>95</sup> In the same study, it was shown that DIPEA gives less epimerization than 2,4,6-collidine, while it has previously been shown that the opposite is true for segment condensations.<sup>96</sup> HAPyU was evaluated both with DIPEA and 2,4,6-collidine in the cyclization of **19a** to **20a**, and DIPEA was found to give a slightly higher yield and less epimerization. Compounds **19b-c** could also be cyclized to **20b-c** using this method (Table 5.1, Scheme 5.5). For comparison, HATU was also tested in the cyclization of **19b** to **20b** and was found to be inferior to HAPyU.

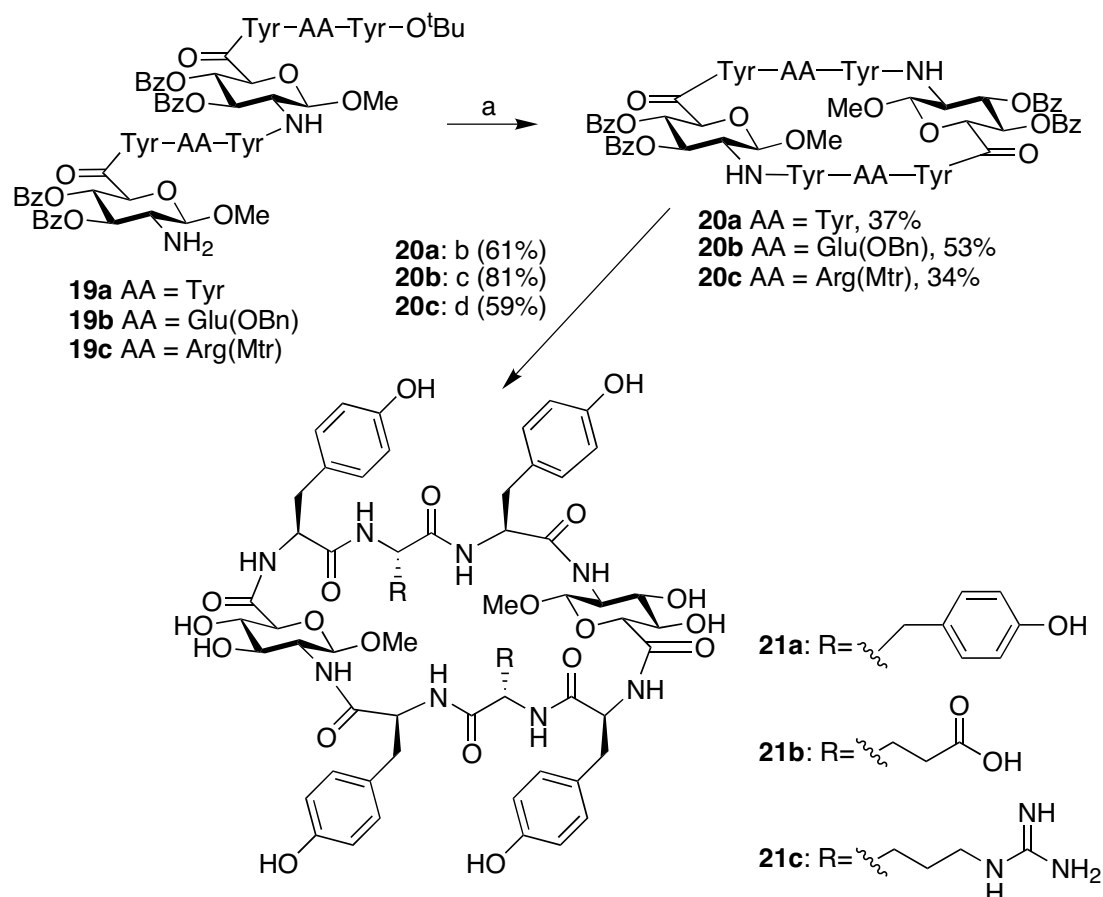
**Table 5.1** Cyclization conditions, yields and epimerization levels.

Starting material	Reagents <sup>a</sup>	Reaction time <sup>b</sup>	Isolated yield <sup>c</sup>	Epimerization
<b>19a</b>	HAPyU/2,4,6-collidine	3 h	35%	5% isolated
<b>19a</b>	HAPyU/DIPEA	1 h	37%	not observed
<b>19b</b>	HAPyU/DIPEA	3 h	53%	traces on TLC
<b>19b</b>	HATU/DIPEA	2.5 h	32%	traces on TLC
<b>19c</b>	HAPyU/DIPEA	4 h	34%	traces on TLC

<sup>a</sup>At room temperature in THF with 1 mM concentration of linear starting material.

<sup>b</sup>Reactions were monitored with MALDI-TOF and run until the starting material was consumed.

<sup>c</sup>For both C-deprotection and cyclization.



**Scheme 5.5** Cyclization and deprotection. Reaction conditions: (a) <sup>i</sup>TFA, Et<sub>3</sub>SiH, CH<sub>2</sub>Cl<sub>2</sub>, 3-4 h; <sup>ii</sup>HAPyU, DIPEA, THF, 1-4 h, see Table 5.1; (b) NaOMe/MeOH, 24 h; (c) <sup>i</sup>HCOOH, Pd black, MeOH, 15 min; <sup>ii</sup>NaOMe/MeOH, 24 h; (d) <sup>i</sup>TFA, PhSMe, 24 h; <sup>ii</sup>NaOMe/MeOH, 24 h.

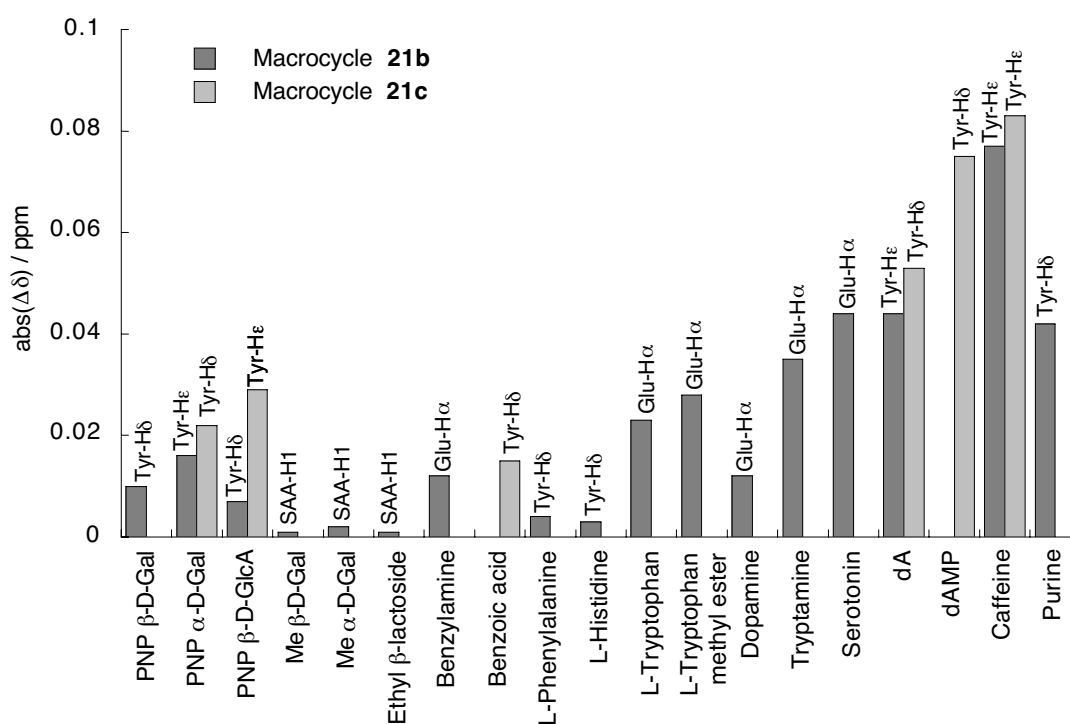
At this point in the synthesis, only deprotection of macrocycles remained. Macrocycle **20a** was treated with NaOMe in MeOH to give **21a** in 61% yield. The benzyl esters of macrocycle **20b** were cleaved using catalytic transfer hydrogenation with palladium black and formic acid,<sup>97</sup> and the benzoates were then cleaved with NaOMe in MeOH to afford **21b** in 81% yield. Macrocycle **20c** was first treated with neat TFA with thioanisole as a scavenger<sup>98</sup> to cleave the Mtr group, and the crude product was then treated with NaOMe in MeOH to afford **21c** in 59% yield after HPLC purification.

The deprotected macrocycles **21a-c** all gave well resolved <sup>1</sup>H NMR spectra at room temperature and exhibited the expected spectra for symmetrical compounds. The spectrum of macrocycle **21c** in D<sub>2</sub>O showed signs of a slow conformational exchange in that smaller additional peaks were seen at 2.81 ppm and 2.56 ppm as well as overlapping peaks at 3.74 ppm and in the aromatic region. The signals coalesced when a sample in DMSO-d<sub>6</sub> was heated to 150°C.

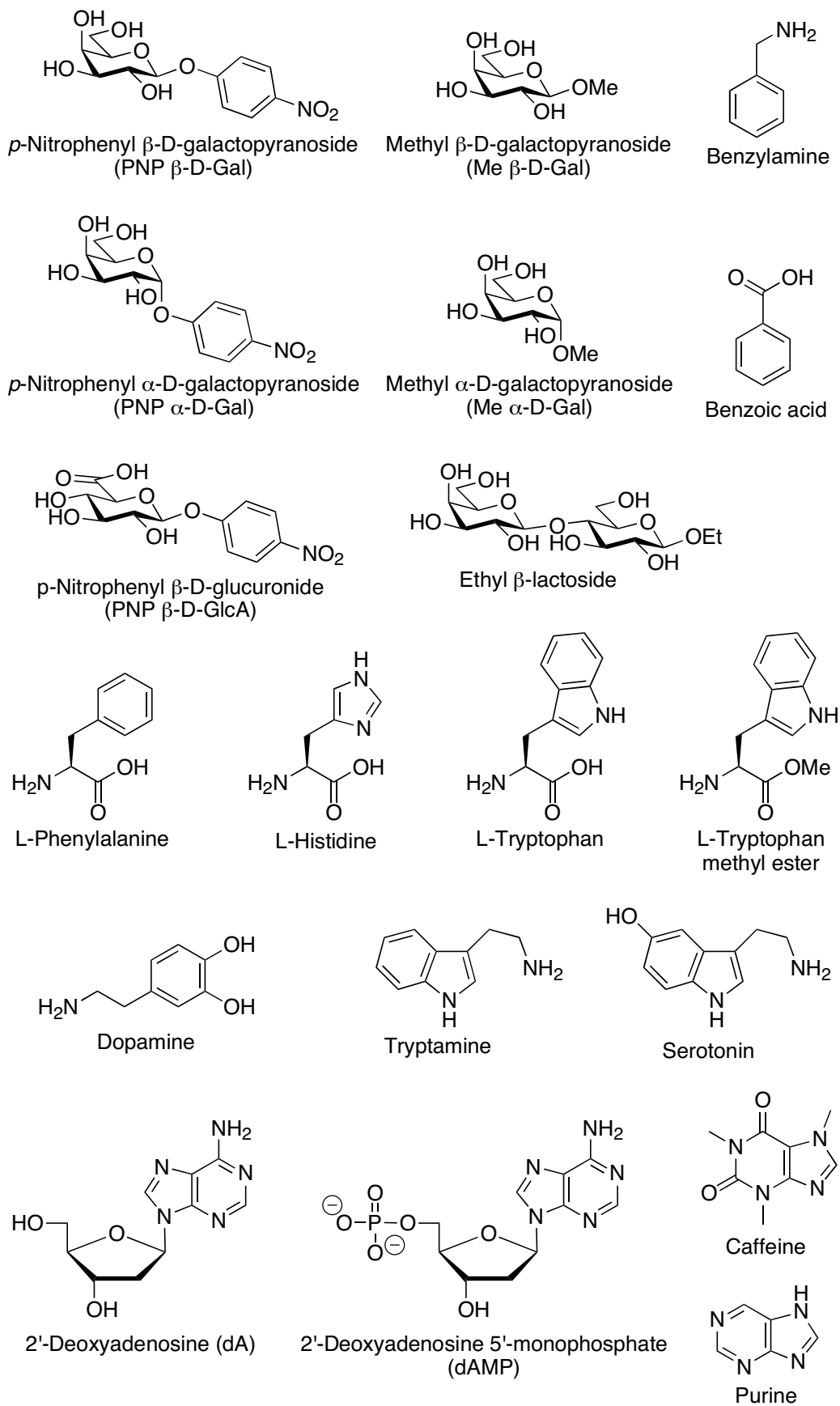


### 5.3 Evaluation as artificial receptors

The all-tyrosine macrocycle **21a** was not soluble in water and its binding properties in other solvents were not studied. Macrocycles **21b-c** were soluble in water and were screened using  $^1\text{H}$  NMR measurements against a number of putative ligands: *p*-nitrophenyl glycosides, nucleotides, aromatic amino acids, aromatic amines and purines. NMR spectra of the macrocycles at 0.5 mM concentration were compared to NMR spectra of mixtures that also contained 20 mM of a ligand in order to find complexation-induced changes in the chemical shifts (Figures 5.2 and 5.3).

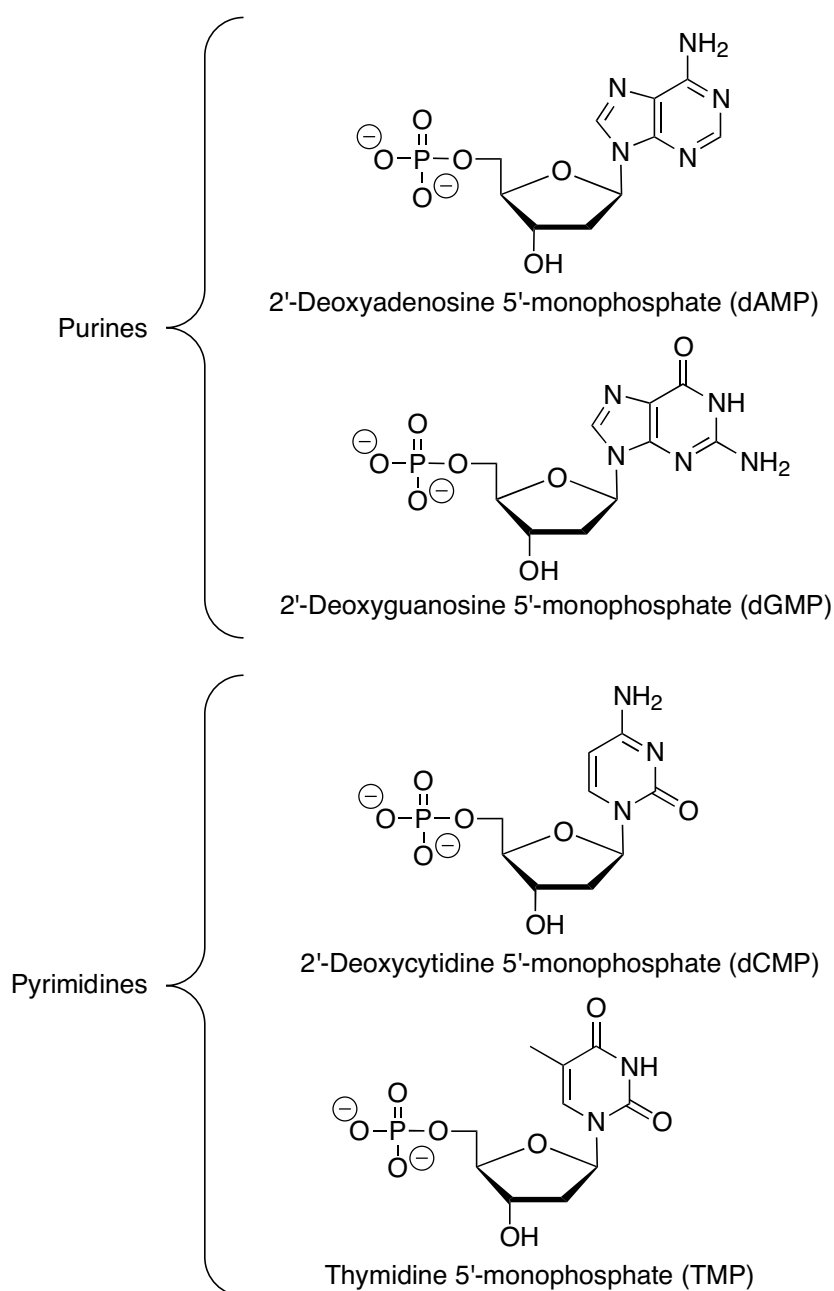


**Figure 5.2** Largest observed absolute changes in chemical shift in macrocycles **21b-c** when putative ligands were added (0.5 mM receptor and 20 mM ligand in 100 mM phosphate buffer, pD 7.2). The observed receptor signals are noted on top of the bars.

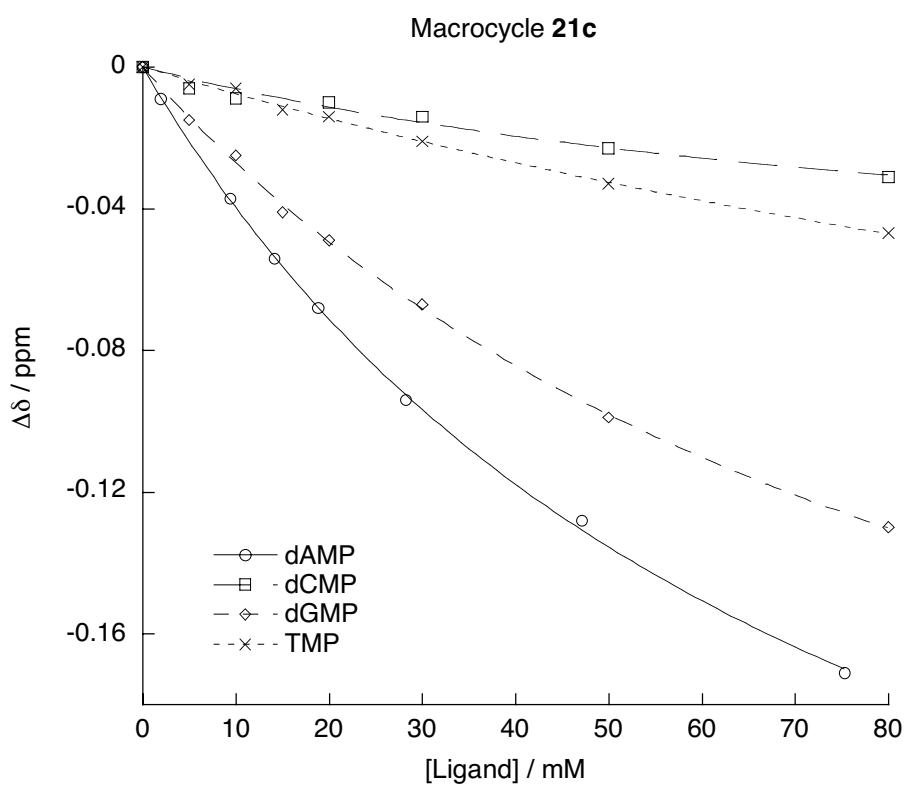
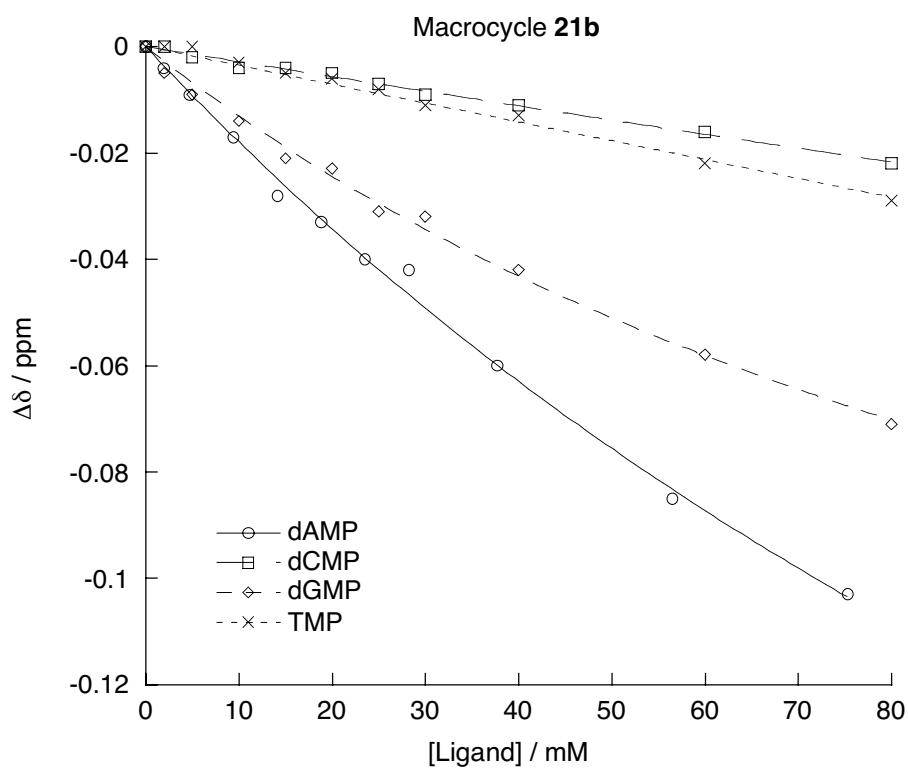


**Figure 5.3** Structures of compounds used in the screening experiments.

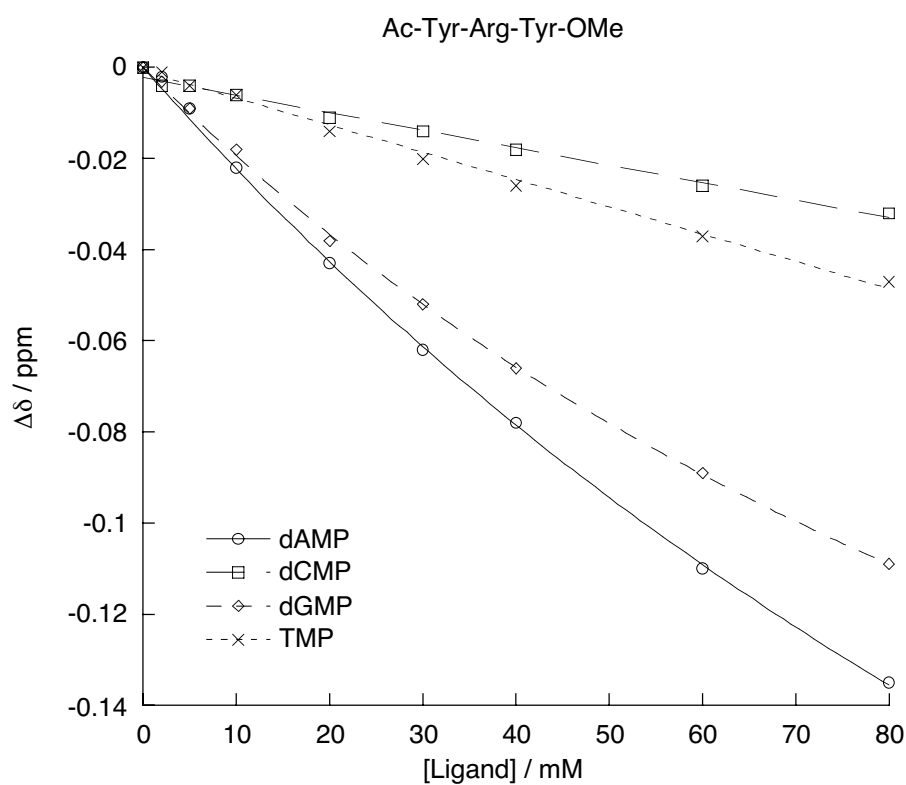
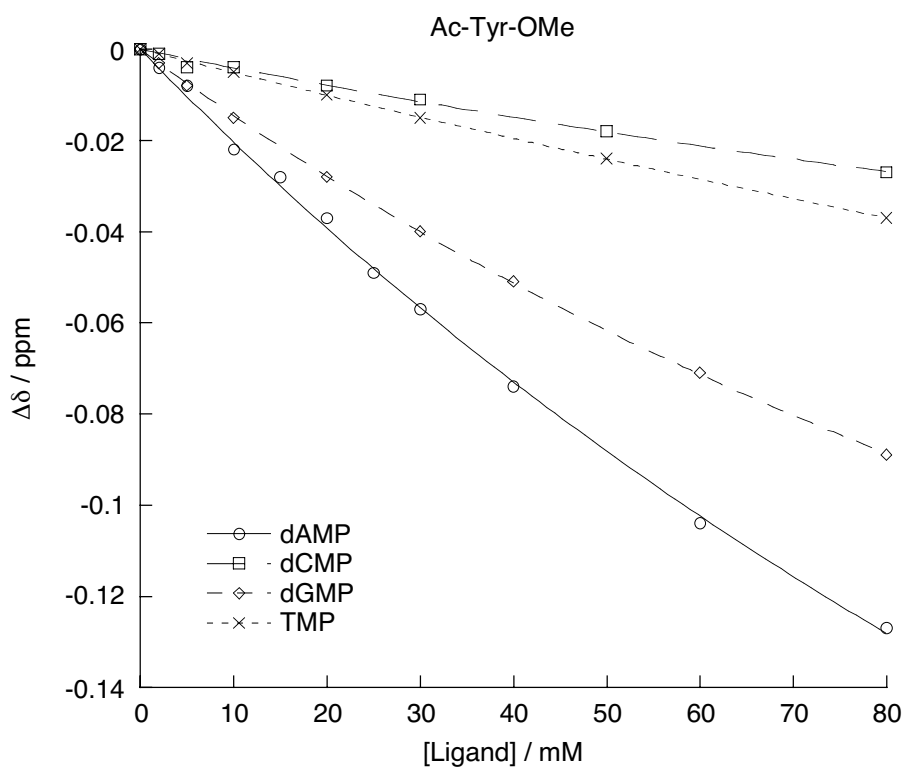
The screening experiments showed large complexation-induced shifts with the purine nucleotides dA and dAMP, mainly in the aromatic signals. To investigate the affinity for nucleotides further, **21b-c** were titrated with the four deoxynucleotides dAMP, dCMP, dGMP and TMP (Figure 5.4) and the changes in chemical shift were plotted against the nucleotide concentration (Figure 5.5). For comparison, Ac-Tyr-OMe and Ac-Tyr-Arg-Tyr-OMe were also titrated with the four deoxynucleotides (Figure 5.6) and binding constants for all titrations were obtained by fitting the curves to a 1:1 binding isotherm<sup>99,100</sup> (Table 5.2).



**Figure 5.4** The deoxynucleotides dAMP, dCMP, dGMP and TMP.



**Figure 5.5** Titration curves (Tyr-H<sup>δ</sup>) of macrocycles **21b-c** (0.5 mM in 100 mM phosphate buffer, pD 7.2) with the deoxynucleotides dAMP, dCMP, dGMP and TMP. The nucleotides were added as sodium salts to ensure a constant pD.



**Figure 5.6** Titration curves (Tyr-H<sup>δ</sup>) of Ac-Tyr-OMe and Ac-Tyr-Arg-Tyr-OMe (0.5 mM in 100 mM phosphate buffer, pD 7.2) with the deoxynucleotides dAMP, dCMP, dGMP and TMP. The nucleotides were added as sodium salts to ensure a constant pD.

**Table 5.2** Binding constants<sup>a</sup> for macrocycles **21b-c**, Ac-Tyr-OMe and Ac-Tyr-Arg-Tyr-OMe with dAMP, dCMP, dGMP and TMP (100 mM phosphate buffer, pD 7.2).

Host	dAMP	dCMP	dGMP	TMP
<b>21b</b>	4-6	<3 <sup>b</sup>	4-5	<3 <sup>b</sup>
<b>21c</b>	10-14	<3 <sup>b</sup>	10-11	<3 <sup>b</sup>
Ac-Tyr-OMe	4	<3 <sup>b</sup>	4-5	<3 <sup>b</sup>
Ac-Tyr-Arg-Tyr-OMe	4-5	<3 <sup>b</sup>	4-7	<3 <sup>b</sup>

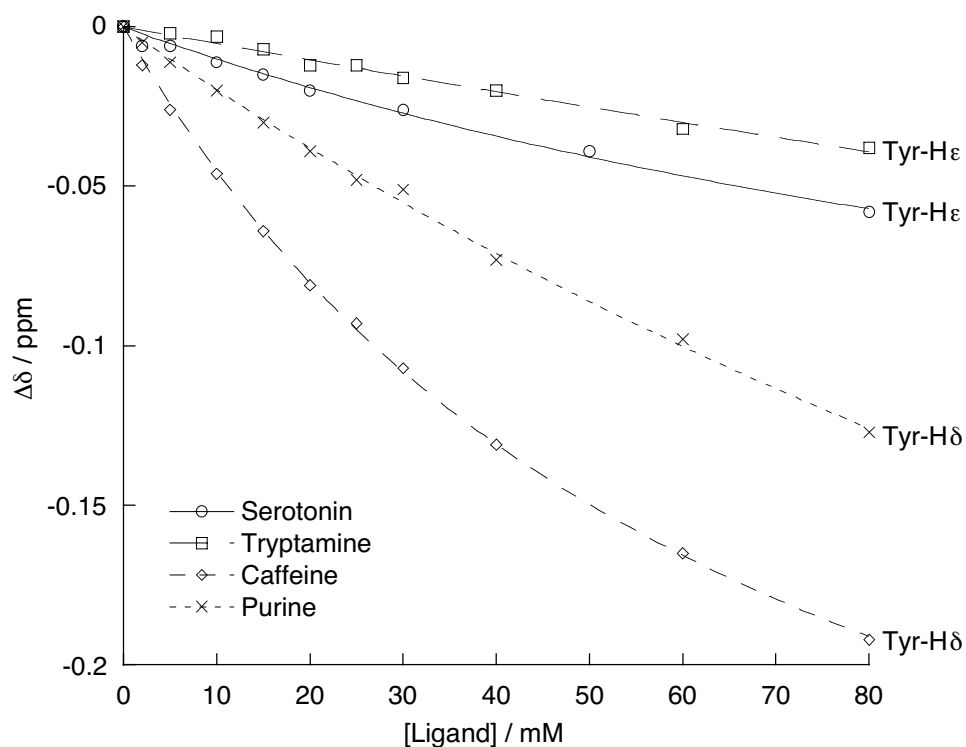
<sup>a</sup>Based on chemical shift changes of the aromatic protons.

<sup>b</sup>Binding curve does not have sufficient curvature for determination of the binding constant.

The titrations revealed that macrocycles **21b-c** bind to dAMP and dGMP, although only weakly. The positively charged macrocycle **21c** binds significantly stronger than the negatively charged macrocycle **21b** as might be expected for molecules binding negatively charged ligands. The control compounds Ac-Tyr-OMe and Ac-Tyr-Arg-Tyr-OMe also bind, but more weakly. Interactions with the purine deoxynucleotides dAMP and dGMP are in all cases stronger than interactions with the pyrimidine deoxynucleotides dCMP and TMP, which bind too weakly for reliable determination of the binding constants. A 1:1 stoichiometry was assumed in all cases since the weak affinity and small shift changes precluded determination of the stoichiometry with Job's plots.<sup>100</sup>

The small binding constants and the stronger affinity for purines than for pyrimidines suggest that the association is mainly through aromatic interactions. It is known from self-association studies and from binding experiments with tryptophan and related compounds that aromatic interactions are stronger with the purines than with the pyrimidines.<sup>101,102</sup> Interestingly, Ac-Tyr-Arg-Tyr-OMe did not show enhanced affinity compared to Ac-Tyr-OMe while the Tyr-Arg-Tyr-containing macrocycle **21c** did. This means that the increased binding strength found with macrocycle **21c** cannot be attributed solely to the presence of the charged arginine side chains. Instead, the stronger binding suggests that the nucleotides either interact with several regions of the macrocycle or that the macrocycle preorganizes the Tyr-Arg-Tyr peptide in a better conformation for binding the nucleotides.

Macrocycle **21b** was also titrated with some of the other putative ligands that had caused significant complexation-induced shift changes and was found to bind weakly to serotonin ( $K_a=6-7 \text{ M}^{-1}$ ) and caffeine ( $K_a=8-15 \text{ M}^{-1}$ ) while interactions with tryptamine ( $K_a<3 \text{ M}^{-1}$ ) and purine ( $K_a<3 \text{ M}^{-1}$ ) were significantly weaker (Figure 5.7).

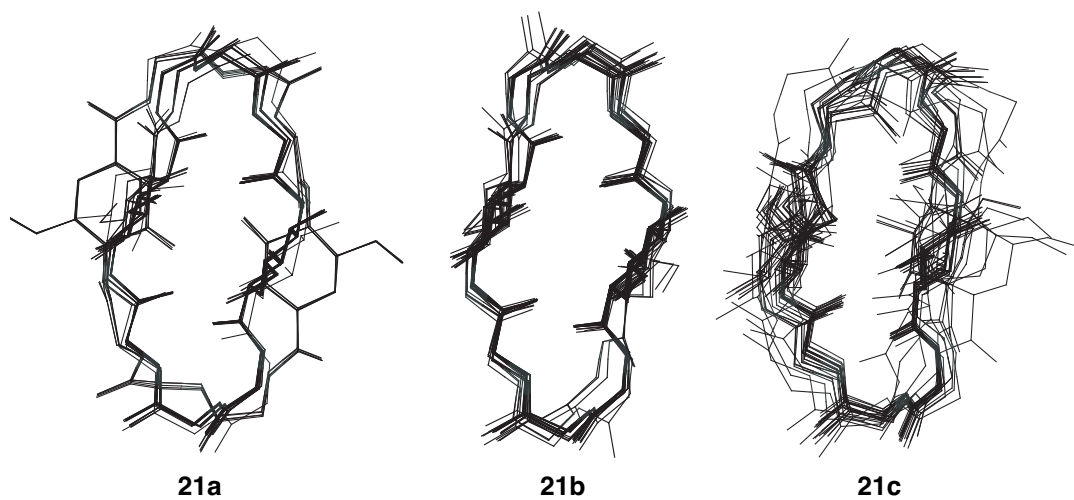


**Figure 5.7** Titration curves (Tyr-H<sup>δ</sup>) of macrocycle **21b** (0.5 mM in 100 mM phosphate buffer, pD 7.2) with serotonin, tryptamine, caffeine and purine. Serotonin and tryptamine were added as hydrochloride salts to ensure a constant pD.

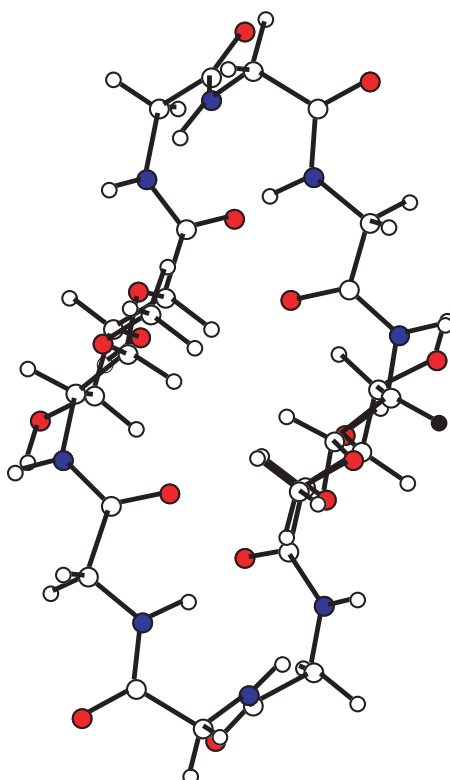
## 5.4 Computational studies

The conformations of macrocycles **21a-c** were calculated using Monte-Carlo conformational searches in MacroModel 8.5 (MMFFs force field with water as solvent, 20,000 steps, all backbone torsions were selected for random variation). The calculations resulted in 110-240 conformers within 5 kcal/mol of the global minimum for each macrocycle (Figure 5.8, only the first 25 conformers for each macrocycle are shown).

Although there was some variation among the low-energy conformers, a closer analysis revealed that they shared similar characteristics. The calculated low-energy conformers for the macrocycles **21a-c** were mostly twisted, oblong structures with extended sugar amino acids and turns formed by the tripeptides (Figure 5.9). Axial hydrogen atoms in the two sugar amino acid residues were facing each other in most of the low-energy conformations, but occasionally a sugar amino acid had rotated to form a hydrogen bond to another part of the molecule. No common pattern in the hydrogen bonding could be discerned within the tripeptides.



**Figure 5.8** Superimposed backbones of the 25 conformers with lowest energy from each of the conformational searches on **21a-c**. Hydrogen atoms have been omitted for clarity.



**Figure 5.9** Typical backbone conformation of macrocycles **21a-c**. The example shown is the global minimum found for macrocycle **21a** (side chains omitted for clarity).



The sugar amino acids in the calculated low-energy conformers are in the  ${}^4C_1$  conformation, which is in agreement with the  ${}^3J_{\text{HH}}$  coupling constants. The sugar amino acids in macrocycle **21c** deviated from the  ${}^4C_1$  conformation in many cases, but since the observed  ${}^3J_{\text{HH}}$  coupling constants in the NMR spectrum of **21c** do not differ markedly from the coupling constants in **21a-b**, this is likely due to an artefact in the calculations induced by a strong hydrogen bond between neighbouring arginine and tyrosine side chains.

## 5.5 Conclusions

The synthetic targets were reached, but unfortunately only weak binding was found with the macrocycles **21b-c**. The best binding constants were around  $10 \text{ M}^{-1}$  while binding constants for nucleotide receptors often are in the range  $10^3\text{-}10^5 \text{ M}^{-1}$ .<sup>103-107</sup>

One reason for the low binding affinity may be the compact conformations of the macrocycles. It was initially hoped that the macrocycles would form a binding site in the centre of the molecules, but the calculations indicate that the only possibility for binding is on the surface of the molecules. More rigid molecules that do not collapse may have a larger potential for being efficient artificial receptors.

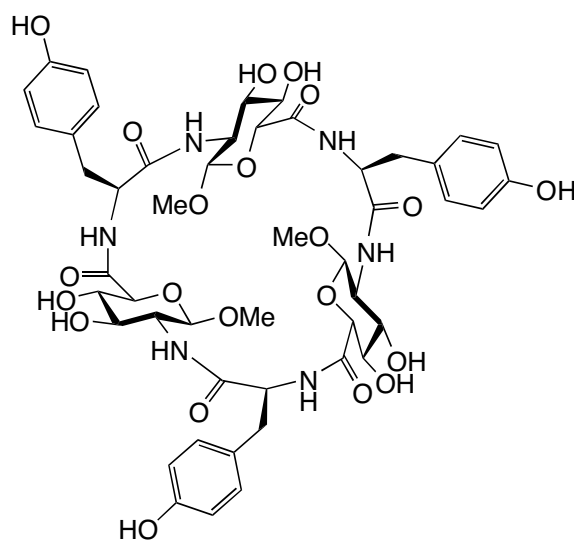
# 6

## $C_3$ -Symmetric Cyclic Peptides With Alternating Tyrosine and Sugar Amino Acid Residues

---

### 6.1 Design considerations

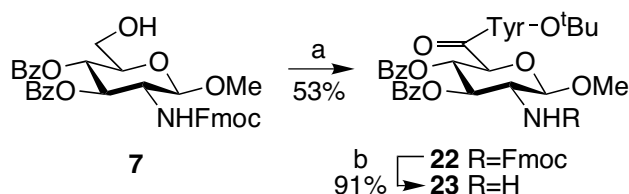
The macrocycles in Chapter 5 consist of two kinds of building blocks, amino acids and a  $\delta$ -sugar amino acid derived from glucosamine. The sugar amino acid is more conformationally restricted than an  $\alpha$ -amino acid due to the pyranoside ring, and it seemed reasonable that increasing the ratio of sugar amino acids to amino acids in the macrocycles would lead to more rigid structures that might form a central cavity instead of collapsing. Preliminary calculations indicated that this was the case, and a  $C_3$ -symmetric molecule with three tyrosine residues and three sugar amino acid residues (Figure 6.1) was selected as the first synthetic target (Paper II).



**Figure 6.1**  $C_3$ -symmetric target molecule.

## 6.2 Synthesis

It was anticipated that this macrocycle could be formed from an orthogonally protected building block consisting of the  $\delta$ -sugar amino acid coupled to tyrosine. Similar chemistry as in the synthesis of the  $C_2$ -symmetric macrocycles (Chapter 5) was employed and sugar amino acid precursor **7** was oxidized and coupled to tyrosine *tert*-butyl ester to give building block **22** (Scheme 6.1). The Fmoc group was then cleaved on part of the material with tetrabutylammonium fluoride and 1-octanethiol<sup>108</sup> to give the N-deprotected building block **23** to be used when elongating the chain.

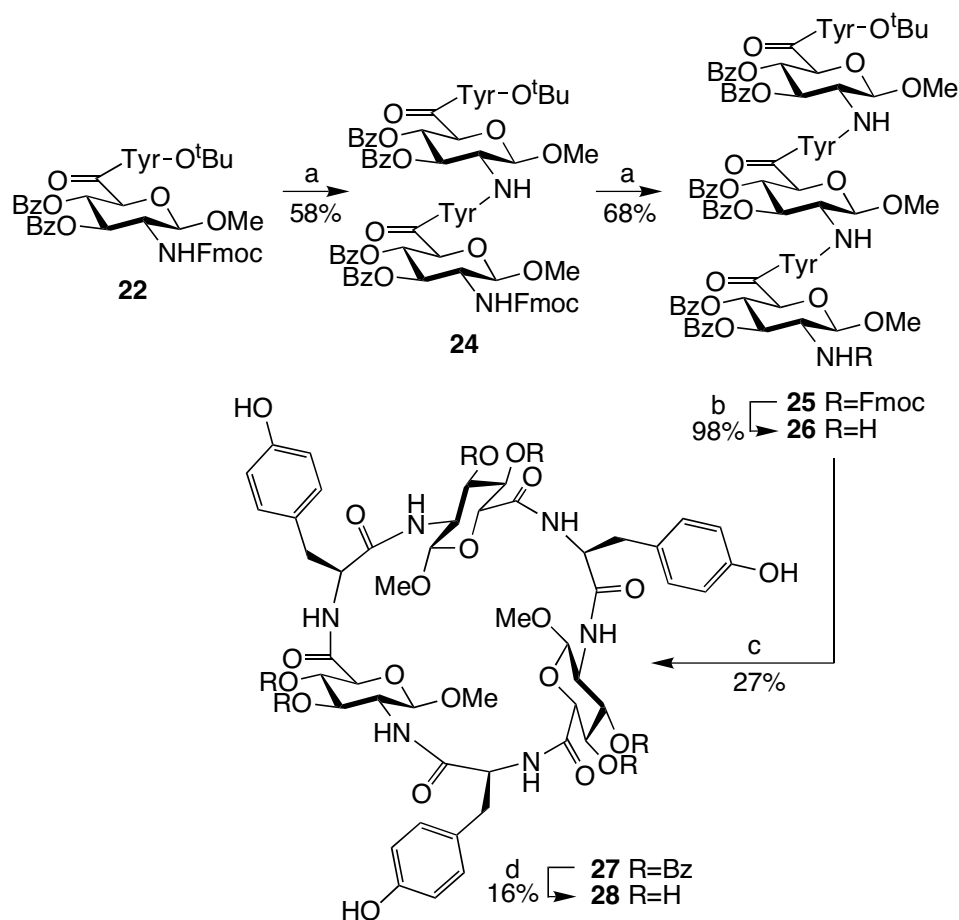


**Scheme 6.1** Synthesis of the building blocks. Reaction conditions:

(a)  $i\text{CrO}_3/\text{H}_2\text{SO}_4(\text{aq})/\text{acetone}$ , 2 h;  $^i\text{H-Tyr-O}^t\text{Bu}/\text{EDC}\cdot\text{HCl}/\text{HOBT}/N\text{-methylmorpholine}/\text{THF}$ , 16 h; (b)  $\text{TBAF}/n\text{-C}_8\text{H}_{17}\text{SH}/\text{THF}$ , sonication, 5 min.

The *tert*-butyl ester of **22** was cleaved with 33% trifluoroacetic acid in  $\text{CH}_2\text{Cl}_2$  with  $\text{Et}_3\text{SiH}$  as a scavenger<sup>93</sup> and the crude product was directly coupled to building block **23** using  $\text{EDC}\cdot\text{HCl}$ ,  $\text{HOBT}$  and  $N$ -methylmorpholine to give **24** (Scheme 6.2). Only 27% of the desired product was obtained along with 19% of epimerized product. As in the synthesis of the  $C_2$ -symmetric macrocycles, epimerization could be reduced by using  $\text{DIC}$  and  $\text{HOBT}$  in the absence of base. With these conditions, 58% of the product and only 6% of the epimer were obtained after separation of the diastereomers.

The linear molecule was now elongated once again. The *tert*-butyl ester of compound **24** was cleaved with  $\text{TFA}/\text{CH}_2\text{Cl}_2/\text{Et}_3\text{SiH}$  and the crude product was coupled with  $\text{DIC}$  and  $\text{HOBT}$  to building block **23** under base-free conditions to obtain **25** in 68% yield. No epimerization was observed in this coupling. The Fmoc group of **25** was then cleaved with  $\text{DBU}$  in the presence of a solid-phase thiol as a scavenger<sup>94</sup> to give **26** in excellent yield. The *tert*-butyl ester was cleaved as before and the crude linear product was successfully cyclized using  $\text{HAPyU}$  and  $\text{DIPEA}$  in  $\text{THF}$  to the  $C_3$ -symmetric macrocycle **27** in 27% yield.



**Scheme 6.2** Synthesis of the macrocycle. Reaction conditions: (a) <sup>i</sup>TFA/Et<sub>3</sub>SiH/CH<sub>2</sub>Cl<sub>2</sub>, 4 h; <sup>ii</sup>**23**/DIC/HOBt/THF, 16 h; (b) DBU/*N*-(2-mercaptoethyl)aminomethyl polystyrene, 6 h; (c) <sup>i</sup>TFA/Et<sub>3</sub>SiH/CH<sub>2</sub>Cl<sub>2</sub>, 4 h; <sup>ii</sup>HAPyU/DIPEA/THF, 3 h; (d) NaOMe/MeOH (2 mM), 5 days.

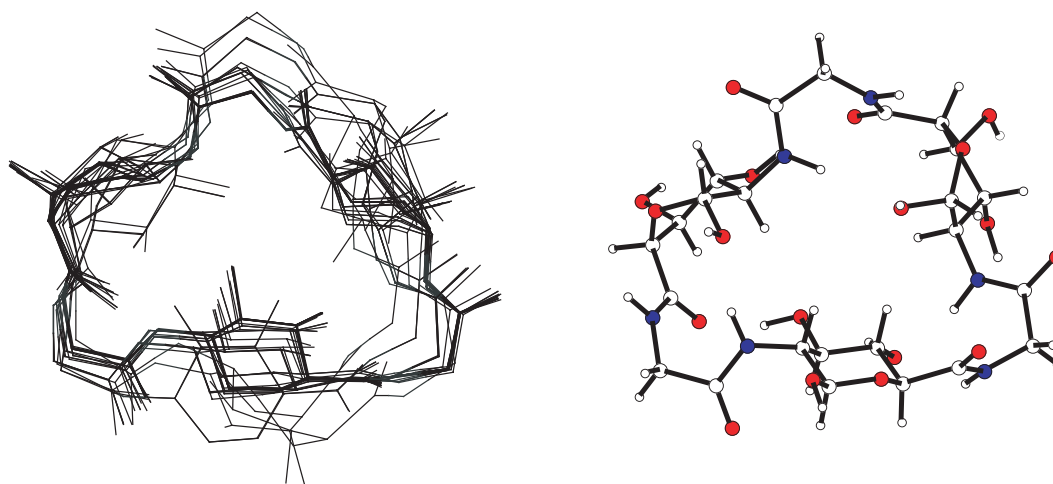
The deprotection of **27** proved to be difficult. Macrocycle **27** was first treated with 10 mM NaOMe in MeOH, which resulted in a number of products. The product mixture was purified using HPLC and 11% of the desired compound **28** was obtained. The main product appeared to be a compound where an elimination had taken place. The high-resolution mass spectrum showed a product that lacked H<sub>2</sub>O when compared to the desired product (HRMS 1061.3606, calculated for **28**+Na 1079.3709) and the <sup>1</sup>H NMR spectrum showed a non-symmetrical compound with an unexpected signal in the olefinic region at ~6 ppm, indicating elimination of water or benzoic acid during the reaction. Deprotection was also attempted at 2 mM NaOMe, and the yield increased somewhat to 16%. Deprotections with Et<sub>3</sub>N, MeNH<sub>2</sub> or 4 Å molecular sieves<sup>109</sup> in MeOH were also attempted, but gave even more side reactions. An attempt was also

made to circumvent the problem by first removing all protective groups from compound **25** and then trying to cyclize the unprotected linear material using HAPyU/DIPEA in DMF, but no product could be detected after the reaction.

### 6.3 Computational studies

It was observed that the  $^3J_{\text{HH}}$  coupling constants in the pyranoside rings in the sugar amino acid residues decrease from 8-10 Hz to 7-8 Hz when the linear molecule **26** is cyclized to macrocycle **27**. This indicates that the  $^4C_1$  chair conformation no longer is the only major conformation of the sugar amino acids, and a Monte-Carlo conformational search in MacroModel 8.5 was carried out on the deprotected macrocycle **28** to calculate the conformation of the molecule (MMFFs force field with water as solvent, 20,000 steps, all backbone torsions were selected for random variation).

The conformational search resulted in 132 conformers within 5 kcal/mol of the global minimum (Figure 6.2) and also showed the presence of conformations other than  $^4C_1$ . The calculated low-energy conformers all had only one of the three sugar amino acid residues in the chair conformation with the other two either in the boat or skew conformations. It thus appears that the macrocycle is strained and the sugar amino acids are forced out of the  $^4C_1$  conformation, which may also be the reason for the difficulties experienced in the deprotection of the macrocycle.



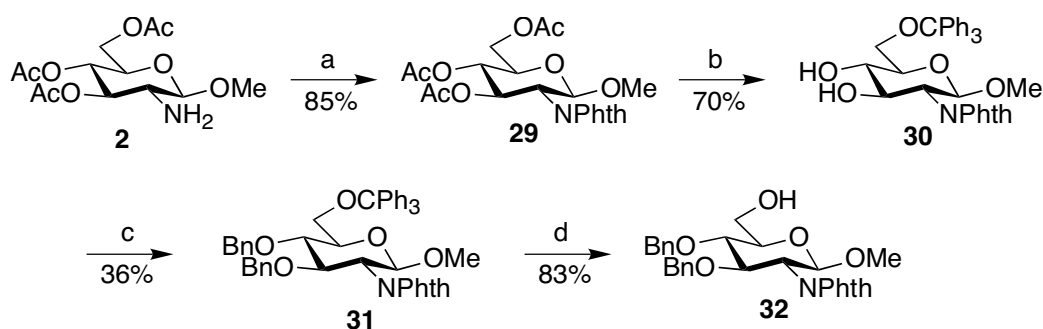
**Figure 6.2** Superimposed backbones of the 25 conformers with lowest energy from the conformational search on **28** (left) and the global minimum found for **28** (right). Side chains and methoxy groups have been omitted for clarity.

## 6.4 Outline of an improved synthesis

### 6.4.1 Protective groups that can be cleaved by hydrogenolysis

The synthesis of the protected macrocycle **27** worked well, but the deprotection was difficult. One way to solve this may be to use protective groups that can be cleaved with hydrogenolysis under neutral conditions. For this purpose, a synthesis was developed towards an analogue of **7** where the carbohydrate hydroxyl groups are protected as benzyl ethers instead of benzoates (Paper IV).

Methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy- $\beta$ -D-glucopyranoside **2** was protected with the phthalimido group by treating it with phthalic anhydride followed by  $\text{Ac}_2\text{O}$  to close the phthalimide (Scheme 6.3) and compound **29** was obtained in 85% yield after crystallization. This compound has been prepared previously using various methods,<sup>110-116</sup> but the phthalimide has always been introduced prior to the formation of the methyl glycoside. The acetate groups of **29** were cleaved using acidic transesterification and the crude product was dissolved in pyridine and allowed to react with chlorotriphenylmethane to afford **30** in 70% yield. The hydroxyl groups of **30** were then protected as benzyl ethers using  $\text{BnBr}$ ,  $\text{NaH}$  and  $\text{Bu}_4\text{NBr}$  in DMF to give **31**. Finally, the triphenylmethyl ether was cleaved using  $\text{HBr}$  in  $\text{AcOH}$  to give sugar amino acid precursor **32** in 83% yield.

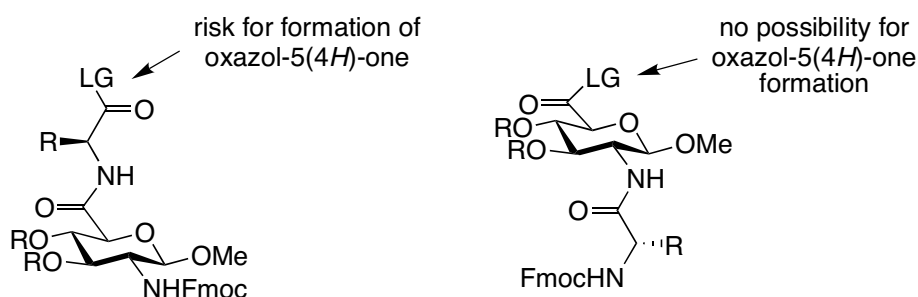


**Scheme 6.3** Synthesis of *O*-benzyl protected sugar amino acid precursor. Reaction conditions: (a)  $^i\text{Phthalic anhydride}/\text{pyridine}/\text{CH}_2\text{Cl}_2$ , 24 h;  $^{ii}\text{Ac}_2\text{O}/\text{pyridine}/\text{CH}_2\text{Cl}_2$ , 24 h; (b)  $^i\text{HCl}/\text{MeOH}$ , 24 h;  $^{ii}\text{Chlorotriphenylmethane}/\text{pyridine}$ , 85°C, 3.5 h; (c)  $\text{BnBr}/\text{NaH}/\text{Bu}_4\text{NBr}$ , 2.5 h, 0°C; (d)  $\text{HBr}/\text{AcOH}$ , 3 min.

Unfortunately, the benzylation yield is currently too low for this to be a truly efficient synthesis. It is known that the phthalimido group is sensitive to bases,<sup>117</sup> but since a compound that only differed in the anomeric group could be benzylated in 88% yield<sup>118</sup> there should be room for improvement.

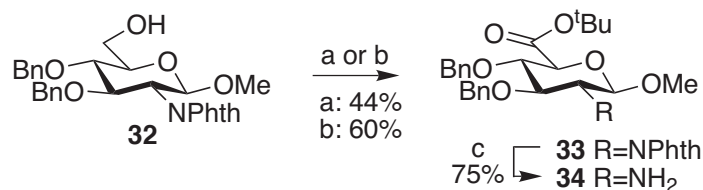
## 6.4.2 Reducing the risk of epimerization

In the synthesis of the  $C_3$ -symmetric macrocycles, there were initially problems with epimerization when couplings were made at the carboxylic acid of tyrosine linked to a sugar amino acid. Although this problem could be reduced by choosing different coupling conditions, there is a possibility to eliminate the risk of epimerization almost completely by switching places of the sugar amino acid and the amino acid in the building block (Figure 6.3). In this situation, there will no longer be any possibility for oxazol-5(4*H*)-one formation and the epimerization levels can be expected to be very low.



**Figure 6.3** Reducing the risk for epimerization by switching places of the amino acid and sugar amino acid in the building block.

In order to prepare a building block with the reverse order of sugar amino acid and amino acid, it is necessary to prepare sugar amino acids with protected carboxylic acid functionality and unprotected amino functionality. Accordingly, a synthesis of the *tert*-butyl ester of the  $\delta$ -sugar amino acid was developed (Scheme 6.4).



**Scheme 6.4** Synthesis of the  $\delta$ -sugar amino acid *tert*-butyl ester. Reaction conditions: (a)  $^i\text{CrO}_3/\text{H}_2\text{SO}_4(\text{aq})/\text{acetone}$ , 2 h; (b)  $^{\text{ii}}\text{Boc}_2\text{O}/\text{DMAP}/t\text{-BuOH}/\text{THF}$ , 18 h; (c)  $\text{CrO}_3/\text{pyridine}/\text{Ac}_2\text{O}/t\text{-BuOH}/\text{CH}_2\text{Cl}_2/\text{DMF}$ , 20 h; (c)  $\text{H}_4\text{N}_2 \cdot \text{H}_2\text{O}/\text{EtOH}$ , reflux, 3 h.

The benzyl protected sugar amino acid precursor **32** could be oxidized using Jones' reagent as before and then directly transformed into the *tert*-butyl ester using  $\text{Boc}_2\text{O}/\text{DMAP}^{119}$  to afford the *tert*-butyl ester **33** in 44% yield. However, a better

alternative is to use a one-step procedure with  $\text{CrO}_3$ , *t*-BuOH and  $\text{Ac}_2\text{O}$ <sup>120</sup> that gave the *tert*-butyl ester directly in 60% yield. The phthalimide was then removed with hydrazine to afford the free amine **34** in 75% yield.

## 6.5 Conclusions

The  $C_3$ -symmetric macrocycle could be prepared with a 27% yield in the cyclization step even though it turned out to be rather rigid. Instead, it was the deprotection that was the major problem and the deprotected macrocycle was only obtained in 16% yield. The macrocycle was only sparingly soluble in water and its binding properties were not studied.

It is reasonable to believe that the deprotection problem can be circumvented by using protective groups that can be cleaved by hydrogenolysis under neutral conditions. The synthesis can also likely be further improved by reversing the order of sugar amino acid and amino acid in the building block so that the risk of epimerization through the oxazol-5(4*H*)-one mechanism is eliminated. For this purpose, a synthesis of the sugar amino acid *tert*-butyl ester **34** was developed, but unfortunately there was no time to actually use it in the synthesis of a macrocycle.





# 7

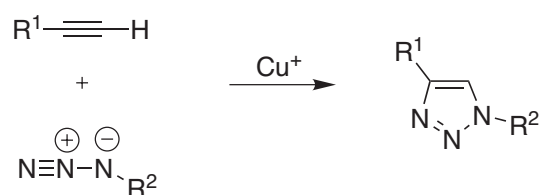
## 1,2,3-Triazole-Containing Macrocycles with $C_2$ -Symmetry

---

### 7.1 Design considerations

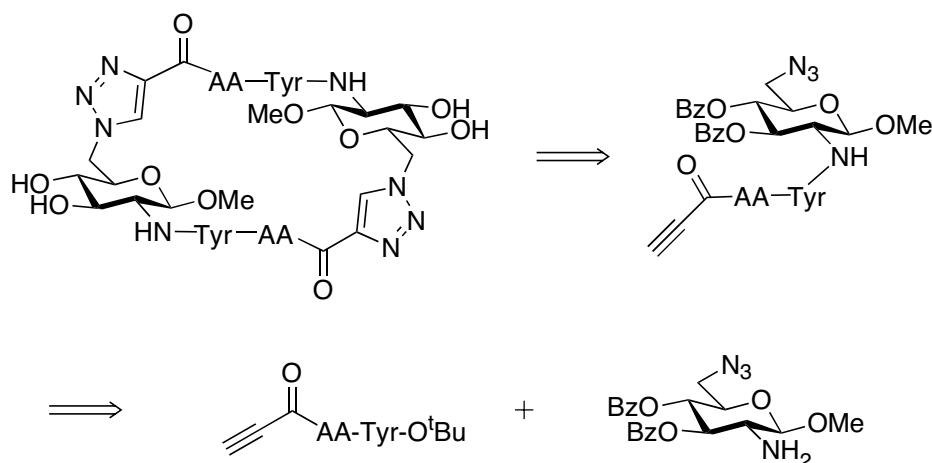
The formation of 1,2,3-triazoles through copper(I)-catalysed 1,3-dipolar cycloaddition of terminal alkynes and azides<sup>121,122</sup> (Figure 7.1) has been shown to be a very versatile reaction<sup>123</sup> that is compatible with most functional groups. Recently, it has also been shown that this reaction can be used to form macrocycles through cyclodimerization of a linear molecule with an azide in one end and an acetylene in the other.<sup>124</sup> The idea to use this cyclodimerization reaction to prepare analogues of the previously prepared macrocycles **21a-c** (Chapter 5) with two tyrosine residues replaced with 1,2,3-triazole units was enticing for several reasons:

- Since a triazole is more rigid than an amino acid residue, it would lead to more rigid molecules that would be more likely to form a central cavity where ligands can bind.
- Aromatic rings would be incorporated directly into the walls of the central cavity instead of only being attached to side chains, thus ensuring that aromatic rings are present in the envisioned binding site in the centre of the macrocycle.
- The formation of the macrocycles can take place in a one-step cyclodimerization which leads to a shorter and more efficient synthesis.



**Figure 7.1** Cu(I)-catalysed 1,3-dipolar cycloaddition of a terminal alkyne and an azide.

The tyrosines at the carboxylate end of the sugar amino acids were good candidates for replacement with 1,2,3-triazoles and a synthesis with cyclodimerization as the key step was designed (Figure 7.2, Paper III).

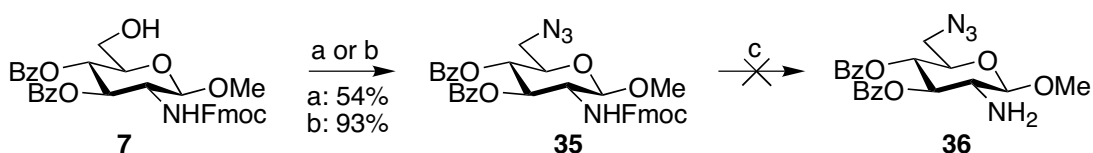


**Figure 7.2** Triazole-containing macrocycles formed by cyclodimerization.

## 7.2 Synthesis

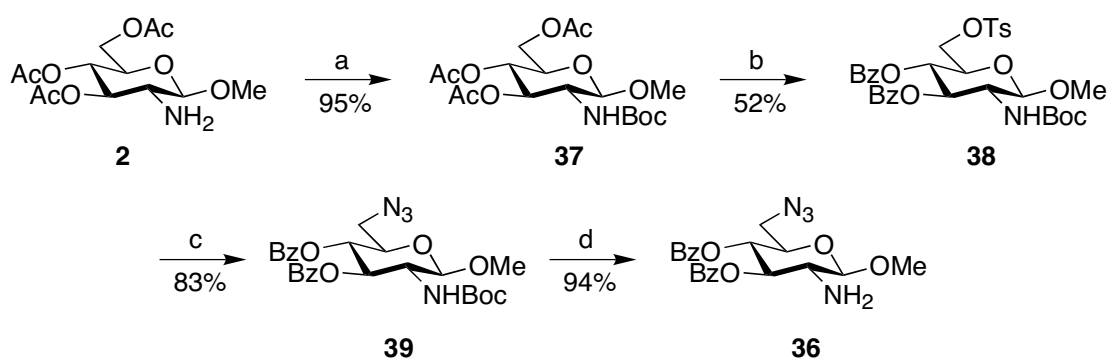
### 7.2.1 Synthesis of the azidoaminosugar

In the synthetic plan, a 6-azido-6-deoxy glucosamine derivative is coupled to an *N*-propionylated dipeptide and the resulting molecule is cyclodimerized. The first step in the synthesis was to prepare this azide, and it was initially hoped that it could be prepared from previously used intermediate **7** using the Mitsunobu reaction (Scheme 7.1). The reaction was performed using either DPPA<sup>125,126</sup> or hydrazoic acid (HN<sub>3</sub>)<sup>127</sup> as the azide source, and azide **35** was obtained in 54% and 93%, respectively. Hydrazoic acid needs to be prepared from sodium azide<sup>128</sup> and is known to be toxic, but was clearly the superior reagent in this case. Deprotection was then attempted with piperidine to obtain **36**, but several products were formed and the deprotected azidoaminosugar could not be obtained in pure form.



**Scheme 7.1** Attempted synthesis of the azidoaminosugar via the Mitsunobu reaction. Reaction conditions: a) DEAD/PPh<sub>3</sub>/DPPA/THF, 3 h; (b) DEAD/PPh<sub>3</sub>/HN<sub>3</sub>/THF, 40 h; (c) piperidine, CH<sub>2</sub>Cl<sub>2</sub>, 30 min.

Instead of trying to optimize the deprotection step, a new and shorter route to the aminoazidosugar **36** was developed (Scheme 7.2). Previously used intermediate **2** was protected with the Boc group using  $\text{Boc}_2\text{O}$  to obtain **37** in 95% yield after crystallization. The acetates were removed with  $\text{NaOMe}$  in  $\text{MeOH}$  and the crude product was dissolved in  $\text{CH}_2\text{Cl}_2/\text{pyridine}$  and first treated with  $\text{TsCl}$  to selectively tosylate the primary hydroxyl group and then with  $\text{BzCl}$  to protect the secondary hydroxyl groups to afford **38** in 52% yield. The tosylate was then reacted with  $\text{NaN}_3$  and 18-crown-6 in  $\text{DMF}$  to give protected azidoaminosugar **39** in 83% yield. The Boc group could be cleanly cleaved using  $\text{TFA}$  in  $\text{CH}_2\text{Cl}_2$  with  $\text{Et}_3\text{SiH}$  as a scavenger<sup>93</sup> to give **36** in 94% yield.



**Scheme 7.2** New route to the azidoaminosugar. Reaction conditions: (a)  $\text{Boc}_2\text{O}/\text{CH}_2\text{Cl}_2$ , 24 h; (b)  $^i\text{NaOMe}/\text{MeOH}$ , 18h;  $^{ii}\text{TsCl}/\text{pyridine}/\text{CH}_2\text{Cl}_2$ , 16 h;  $^{iii}\text{BzCl}/\text{pyridine}/\text{CH}_2\text{Cl}_2$ , 3 h; (c)  $\text{NaN}_3/18\text{-crown-6}/\text{DMF}$ , 60 °C, 24 h; (d)  $\text{TFA}/\text{CH}_2\text{Cl}_2/\text{Et}_3\text{SiH}$ , 3 h.

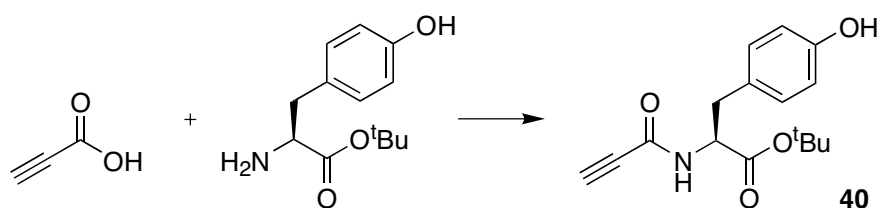
### 7.2.2 Synthesis of the *N*-propiolyl dipeptides

It became known from initial unsuccessful attempts that it was difficult to couple propiolic acid to a peptide using the  $\text{EDC}\cdot\text{HCl}/\text{HOBT}/N\text{-methylmorpholine}$  coupling protocol. In order to find working coupling conditions for propiolic acid, attempts were made to do couplings to *L*-tyrosine *tert*-butyl ester under various conditions (Table 7.1). First, the  $\text{EDC}\cdot\text{HCl}/\text{HOBT}/N\text{-methylmorpholine}$  coupling protocol was tried again. This resulted in a number of products and only 10% of impure *N*-propiolyl-*L*-tyrosine *tert*-butyl ester **40** could be found. The reagents were only partially soluble in THF and to ensure that it was not merely a solubility problem, the reaction was repeated in  $\text{DMF}$  which gave the same results.

A literature survey showed only a limited number of couplings with propiolic acid, and only one example with  $\text{EDC}$  with a moderate yield of 48%.<sup>129</sup>  $\text{DMTMM}$ <sup>130</sup>,  $\text{DCC}$ <sup>131</sup>,  $\text{PyBrOP}$ <sup>132</sup> and  $\text{EEDQ}$ <sup>122</sup> had however been used with good results.  $\text{EEDQ}$  was

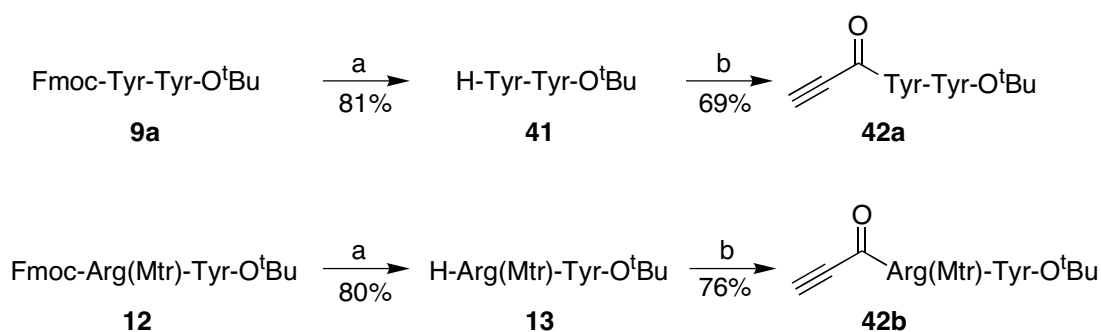
used as the last step in a multi-step sequence and no separate yield for the coupling step was given, but the use of mixed anhydrides seemed promising and it was attempted to prepare the mixed anhydride of propiolic acid using isobutylchloroformate and *N*-methylmorpholine in THF. As soon as the chloroformate was added to the propiolic acid a precipitate was formed, but L-tyrosine *tert*-butyl ester was added nonetheless. The mixture was allowed to stand for 2 h, but no product could be detected. The formation of the mixed anhydride was then attempted using EEDQ. The coupling product **40** was formed in a good yield of 77%, and this method was deemed to be efficient enough for use in the synthesis.

**Table 7.1** Attempts to couple propiolic acid to L-tyrosine *tert*-butyl ester.



Conditions	Yield
EDC•HCl/HOBt/ <i>N</i> -methylmorpholine, THF, 24 h, r. t	~10% (impure)
EDC•HCl/HOBt/ <i>N</i> -methylmorpholine, DMF, 16 h, r. t	~10% (impure)
Isobutylchloroformate/ <i>N</i> -methylmorpholine, THF, 2 h, 0 °C	0%
EEDQ, DMF, 16 h, r. t.	77%

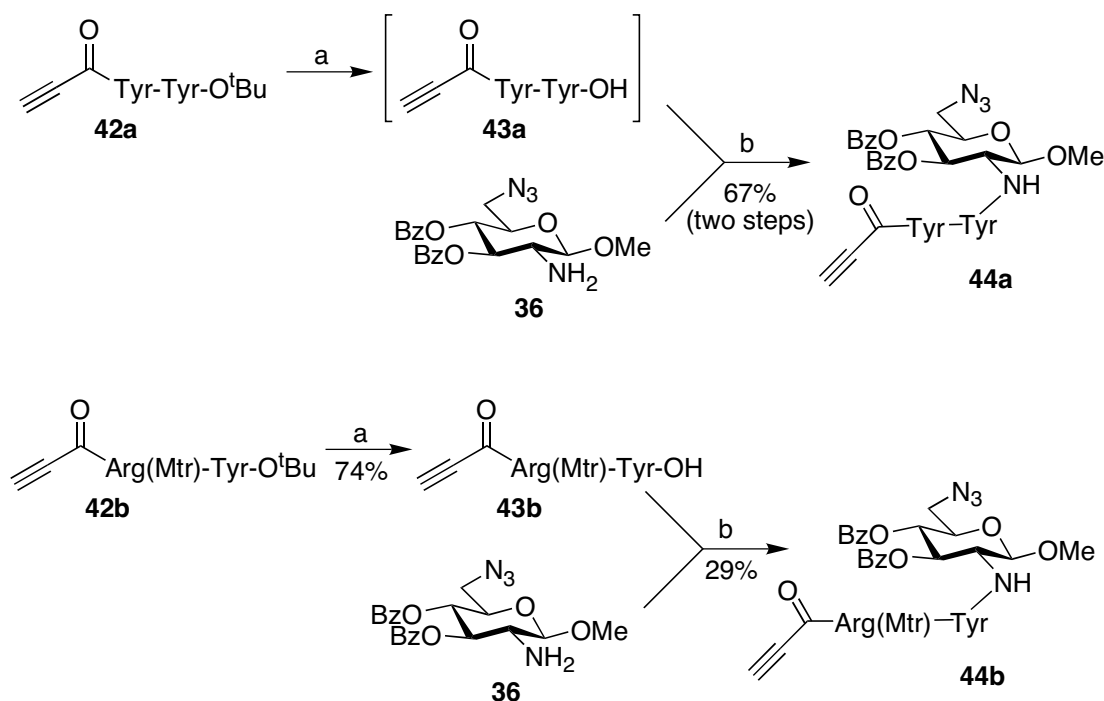
Now that efficient conditions had been found for coupling propiolic acid, the required *N*-propiolyl dipeptides could be prepared (Scheme 7.3). The Fmoc group was cleaved from previously used dipeptides **9a** and **12** to obtain dipeptide *tert*-butyl esters **41** and previously used **13**. These were coupled to propiolic acid using EEDQ in DMF to obtain propiolamides **42a-b**.



**Scheme 7.3** Synthesis of *N*-propiolyl dipeptides **42a-b**. Reaction conditions: (a) Bu<sub>4</sub>NF/1-octanethiol/THF, sonication, 5 min; (b) propiolic acid/EEDQ/DMF, 16 h.

### 7.2.3 Synthesis of the macrocycles

The *tert*-butyl ester of **42a** was cleanly cleaved with TFA in CH<sub>2</sub>Cl<sub>2</sub> with Et<sub>3</sub>SiH as a scavenger<sup>93</sup> and the crude free acid was directly coupled to azidoaminosugar **36** under conditions known to give little epimerization<sup>83</sup> to afford **44a** in 67% yield (Scheme 7.4). The same sequence was attempted with **42b**, but only 18% of **44b** was obtained. Investigation of the crude free acid after *tert*-butyl cleavage showed that the Mtr group was not entirely stable during the reaction even though no cleavage had been observed at the same conditions with other Mtr-protected peptides in the synthesis of the C<sub>2</sub>-symmetric macrocycles (Chapter 5). The product mixture after *tert*-butyl cleavage was separated using chromatography to afford **43b** in 74% yield. The free acid **43b** was then coupled to azidoaminosugar **36** to afford **44b** in 29% yield (21% over both steps). Thus it appears that the partial Mtr cleavage was not the only reason for the low yield, and that the coupling to the arginine-containing peptide is more difficult. Possibly better yields might be obtained with di-CBz protection of the arginine side chain where both terminal nitrogens on the guanidino group are protected.



**Scheme 7.4** Synthesis of linear starting materials for the cyclodimerization. Reaction conditions: (a) TFA/Et<sub>3</sub>SiH/CH<sub>2</sub>Cl<sub>2</sub>, 4 h; (b) DIC/HOBt/THF, 16 h.

Now that the required linear starting materials had been obtained, attempts were made to cyclodimerize **44a** under various conditions (Table 7.2). In the previous cyclodimerization of a similar starting material,<sup>124</sup> CuI and DBU in toluene had been used and these conditions were tried first. The reaction turned out to be sluggish with **44a** at room temperature and only traces of product could be seen using MALDI-TOF analysis of the reaction mixture. When the reaction was heated to 85 °C overnight, the starting material was consumed but no product could be found. The reaction was repeated at 50 °C, and also with DIPEA instead of DBU, but the results were similar.

**Table 7.2** Cyclodimerization attempts with **44a**.

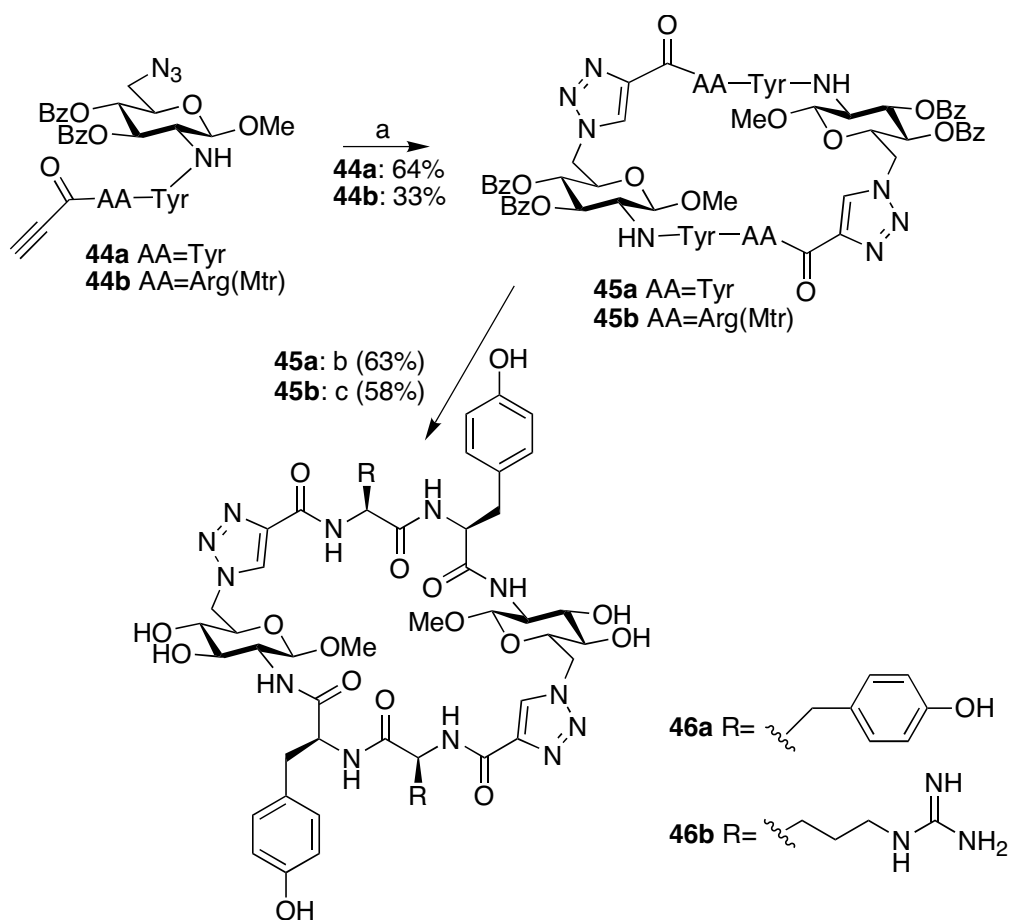
Reagents	Solvent	Conc.	Temperature	Time	Yield
CuI/DBU	toluene	2.5 mM	r.t., then 85 °C	24 h, then 16 h	-
CuI/DBU	toluene	2.5 mM	50 °C	16 h	-
CuI/DIPEA	toluene	2.5 mM	45 °C	6 days	-
CuI/DIPEA	THF	2.5 mM	45 °C	7 days	16%
CuI/DIPEA	THF	0.25 mM	45 °C	28 days	45%
CuI/DIPEA	CH <sub>3</sub> CN	2.5 mM	45 °C	24 h	20%
CuI/DIPEA	CH <sub>3</sub> CN	0.25 mM	45 °C	3 days	64%
CuSO <sub>4</sub> /Na-ascorbate	<i>t</i> -BuOH:H <sub>2</sub> O 4:1	2.5 mM	r.t., then 70 °C	48 h, then 7 days	9%

One reason for the sluggish reaction may be the limited solubility of the starting material in toluene, and THF was tried instead. The reaction was still slow, but 16% of cyclodimerized material was obtained after 7 days along with an insoluble compound. The insoluble compound gave broad signals on NMR and is likely to be polymerized starting material. To decrease the polymerization, the reaction was repeated at ten times more dilute conditions. The reaction took four weeks to complete, but the yield increased dramatically to 45%.

The long reaction time was somewhat impractical and further optimizations were made. The reaction was repeated in CH<sub>3</sub>CN and was found to be much faster. Again, the more dilute conditions gave better results and the reaction only needed three days to complete. It has been noted<sup>121</sup> that the CuI-catalysed formation of 1,2,3-triazoles works better in CH<sub>3</sub>CN and it has been proposed<sup>133</sup> that the reason for this is that CH<sub>3</sub>CN coordinates to the Cu(I) ions and prevents oxidation to Cu(II) ions.

The Sharpless conditions<sup>121</sup> where Cu(I) ions are formed from CuSO<sub>4</sub> and sodium ascorbate were also tried, but with the ratio of *t*-BuOH to water increased from 1:2 to 4:1 in order to increase the solubility of the starting material. The reaction was very sluggish at room temperature, and although the starting material was consumed when the reaction mixture was heated to 70 °C for a week, the product was obtained in only 9% yield.

Cyclodimerization could thus be performed efficiently with CuI and DIPEA in CH<sub>3</sub>CN. The arginine-containing linear precursor **44b** could also be cyclodimerized to **45b** in 33% yield under these conditions (Scheme 7.5), but the reaction was slower and needed 10 days to complete. The macrocycles were then deprotected. Macrocycle **45a** was treated with NaOMe in MeOH to afford **46a** in 63% yield. Macrocycle **45b** was first treated with TFA containing 5% thioanisole<sup>98</sup> for 24 h to cleave the Mtr group, and the crude product was then treated with NaOMe in MeOH to cleave the benzoates to afford **46b** in 58% yield. Both deprotected macrocycles were purified using HPLC.

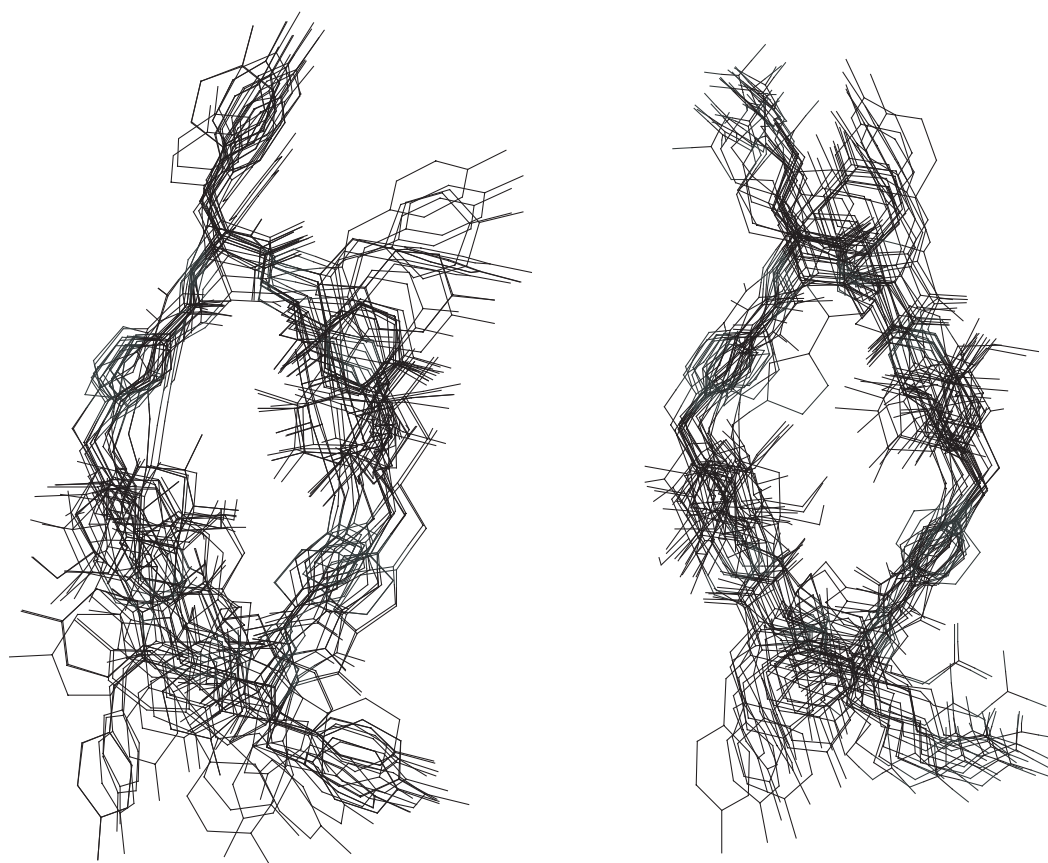


**Scheme 7.5** Cyclization and deprotection. Reaction conditions: (a) CuI/DIPEA/THF, 45 °C; (b) NaOMe/MeOH, 24 h; (c) TFA/PhSMe, 24 h; <sup>iv</sup>NaOMe/MeOH, 48 h.

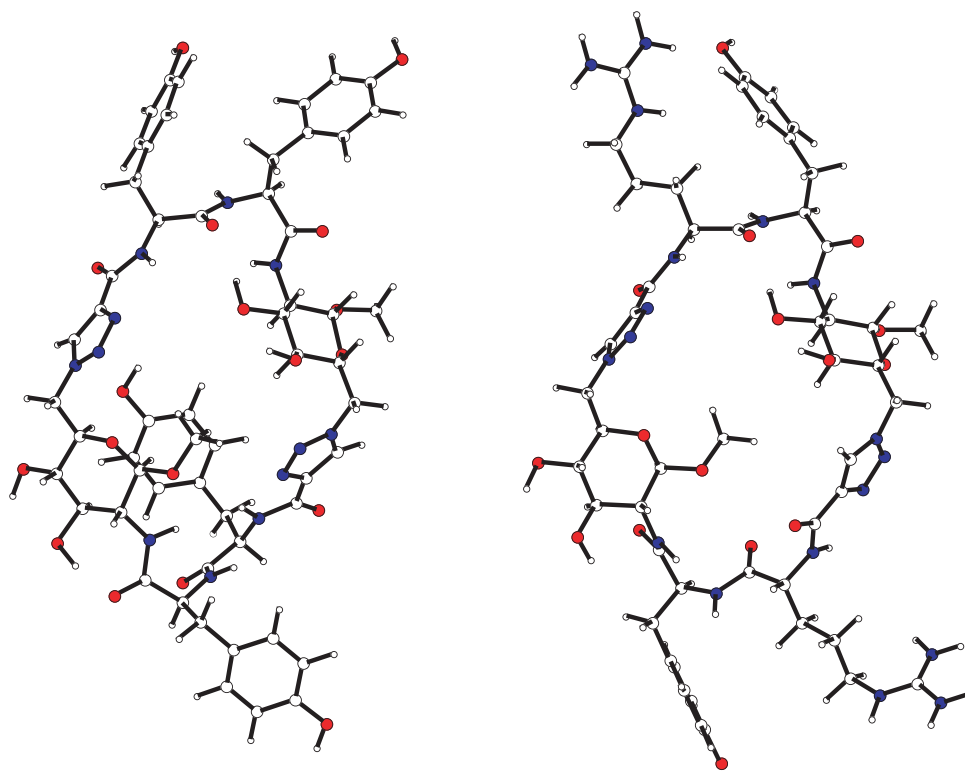


### 7.3 Computational studies

Structures **46a-b** were subjected to conformational searches in MacroModel 8.5 (MMFFs force field in water, all backbone torsions were selected for variation, 20,000 steps). The searches resulted in a number of different conformations with similar energies, and no single dominating conformer could be found (Figure 7.3). However, the overall shape of the conformers was similar and they all had a rather open structure with an accessible core. The global minima for **46a** and **46b** were similar and mainly differed in the orientation of one of the triazole rings (Figure 7.4).



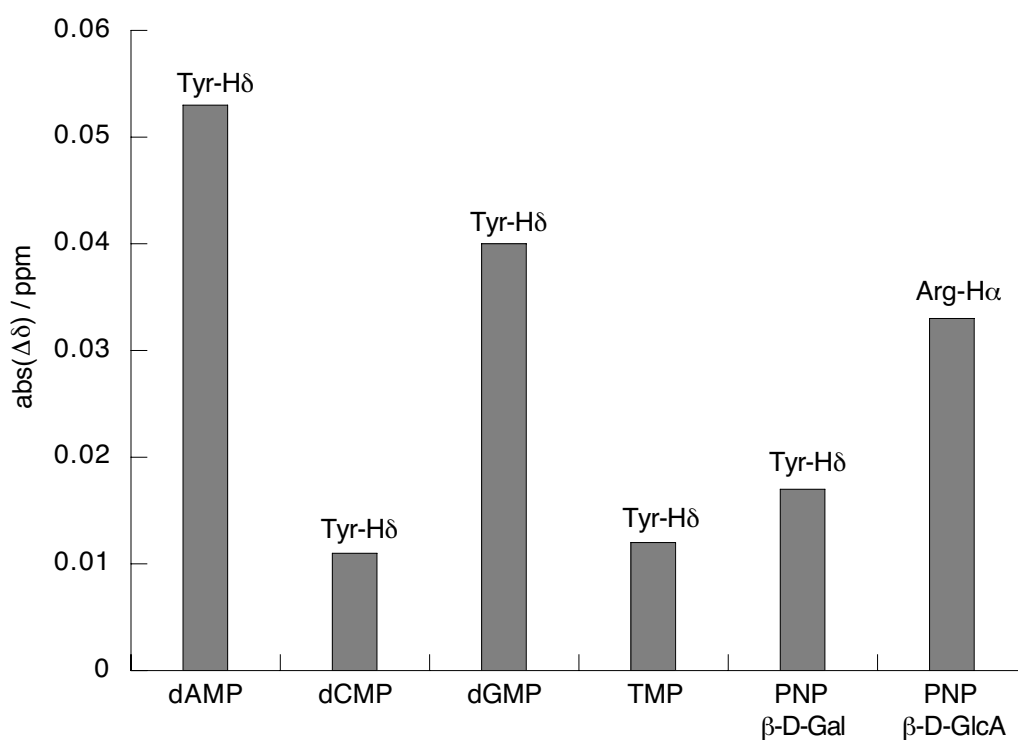
**Figure 7.3** Superimposed structures of the 25 conformers with lowest energy from the conformational searches on **46a** (left) and **46b** (right).



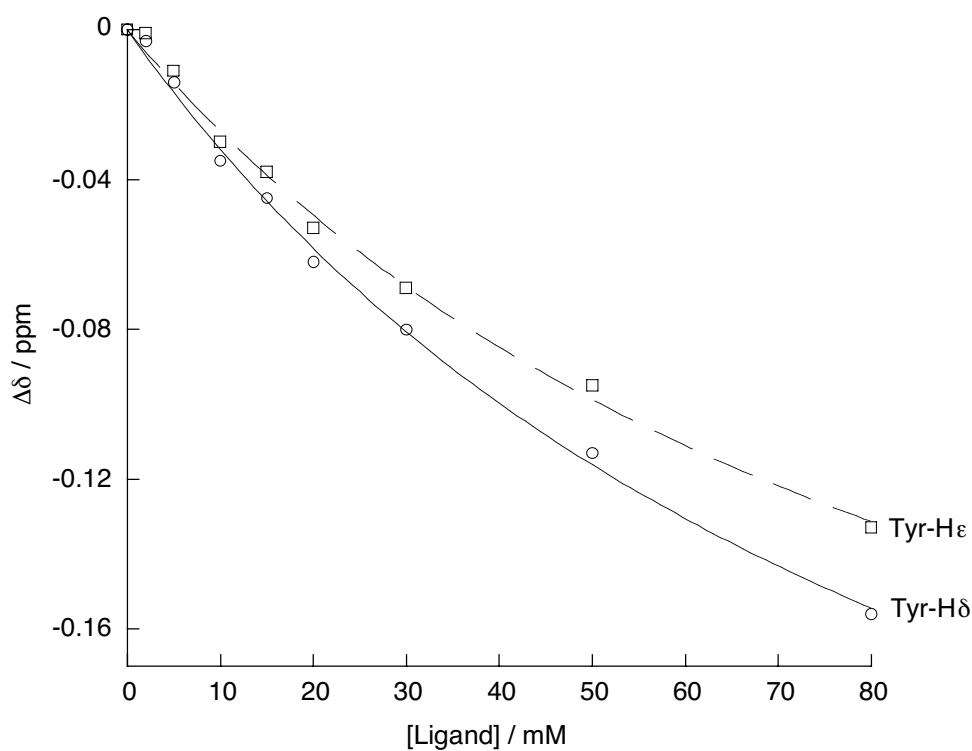
**Figure 7.4** Calculated global minima for **46a** (left) and **46b** (right).

## 7.4 Binding studies

To compare the binding properties of these macrocycles with the binding properties of the similar macrocycles **21b-c** (Chapter 5), macrocycle **46b** was screened against the deoxynucleotides dAMP, dCMP, dGMP and TMP (Figure 7.5). The neutral glycoside *p*-nitrophenyl  $\beta$ -D-galactopyranoside and the negatively charged glycoside *p*-nitrophenyl  $\beta$ -D-glucuronide were also included in the screening experiment. The same pattern was seen with **46b** and the deoxynucleotides as had previously been seen with macrocycle **21b-c**, i.e. the changes in chemical shifts were larger with the purines dAMP and dGMP than with the pyrimidines dCMP and TMP. To compare the affinity, **46b** was titrated with dAMP (Figure 7.6). The resulting binding curve was fitted to a 1:1 binding isotherm<sup>99,100</sup> and the binding constant was found to be  $10 \text{ M}^{-1}$ . The binding constant for **21c** had previously been found to be  $10\text{-}14 \text{ M}^{-1}$ , and macrocycles **21c** and **46b** thus have similar affinity for dAMP. Macrocycle **46b** was also titrated with *p*-nitrophenyl  $\beta$ -D-glucuronide, but no significant binding was found.



**Figure 7.5** Largest observed absolute changes in chemical shift in macrocycle **46b** when putative ligands were added (0.5 mM receptor and 20 mM ligand in 100 mM phosphate buffer, pD 7.2). The observed receptor signal is noted on top of the bars.



**Figure 7.6** Titration curves of **46b** (0.5 mM in 100 mM phosphate buffer, pD 7.2) with dAMP. The nucleotide was added as the sodium salt to ensure a constant pD.

## 7.5 Conclusions

$C_2$ -symmetric macrocyclic carbohydrate/amino acid hybrid molecules can be efficiently prepared using the copper(I)-catalysed addition of acetylenes to azides. Calculations indicate that the strategy to replace two tyrosine residues with triazole units in order to get more rigid macrocycles is working. The arginine-containing macrocycle **46b** was screened against the deoxynucleotides dAMP, dCMP, dGMP and TMP and was found to exhibit the same pattern as its predecessor **21c**, i.e. large changes in chemical shift with the purines dAMP and dGMP and only small changes in chemical shift with the pyrimidines dCMP and TMP. A titration experiment with **46b** and dAMP showed that the binding constant was  $10 \text{ M}^{-1}$ , the same as for **21c**. Although the binding affinity was low in this case, the calculated low-energy conformers do have a relatively open structure and macrocycles of this type may still have a potential for forming binding sites in their centres.



# 8

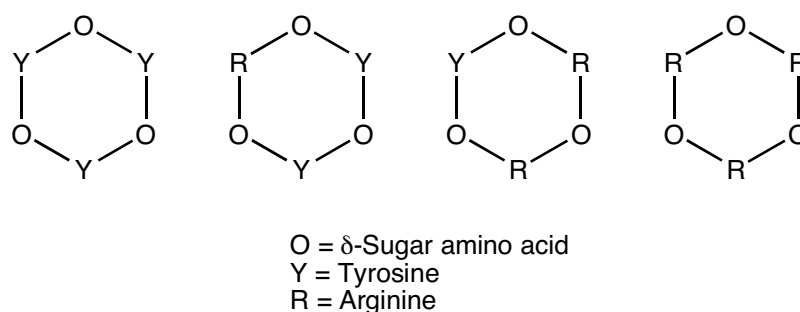
## Summary and Future Perspectives

---

Methods for the preparation of three different types of macrocyclic carbohydrate/amino acid hybrids have been developed. In the design of the first macrocycles (Chapter 5), the hypothesis was that ligands would bind in the centre of the macrocycles. However, only weak interactions with other molecules were obtained ( $K_a \approx 10 \text{ M}^{-1}$  for dAMP, dGMP, serotonin and caffeine), and calculations indicated that the molecules were too flexible to form a central cavity and instead collapsed and formed closed structures. Two different strategies were then pursued to obtain more rigid macrocycles. In the first strategy (Chapter 6), the ratio of sugar amino acids to  $\alpha$ -amino acids was increased and a macrocycle with three sugar amino acid residues alternating with three tyrosine residues was prepared. Calculations indicated that the macrocycle was more rigid, but unfortunately difficulties were encountered in the deprotection of the macrocycle. The obtained macrocycle was only sparingly water-soluble, and macrocycles with other amino acids were not prepared. In the second strategy to obtain more rigid macrocycles, two amino acids in the original macrocycles were replaced with 1,2,3-triazole units (Chapter 7). Calculations indicated that the macrocycles formed open structures with an accessible core, but a titration experiment with dAMP did not show enhanced affinity compared to the original macrocycles.

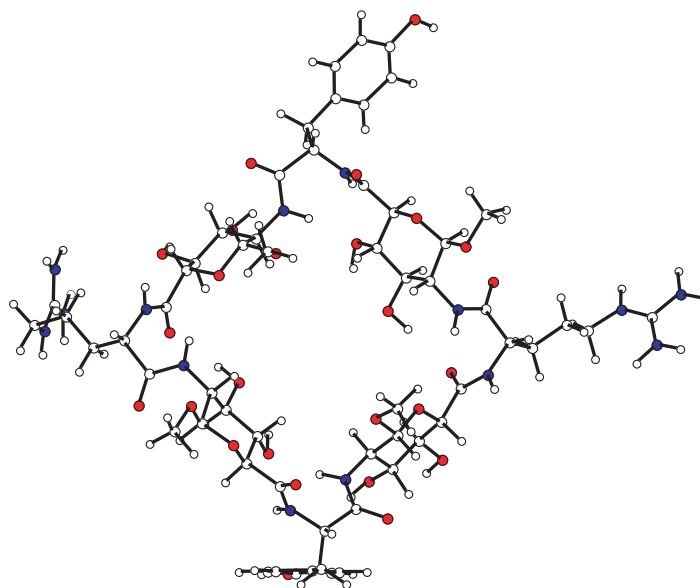
Although the macrocycles that were evaluated in binding studies only interacted weakly with the tested ligands, the results are nevertheless an indication that structures such as these may be interesting receptors, and there are several ways that this research could be continued. The  $C_3$ -symmetric macrocycles with alternating sugar amino acids and  $\alpha$ -amino acids were never evaluated as artificial receptors and further research on this type of macrocycles would be interesting. An *O*-benzyl protected sugar amino acid *tert*-butyl ester was prepared and proposed as a better building block for macrocycles of this type and if the difficulties in the synthesis can be overcome with this new building block,

it would be interesting to prepare all four possible combinations with tyrosine and arginine and compare their binding properties (Figure 8.1). The four combinations could hopefully be prepared from common building blocks in an efficient fashion, and a side-chain protective group for arginine that could also be cleaved by hydrogenolysis, such as the nitro or CBz groups, would have to be used.



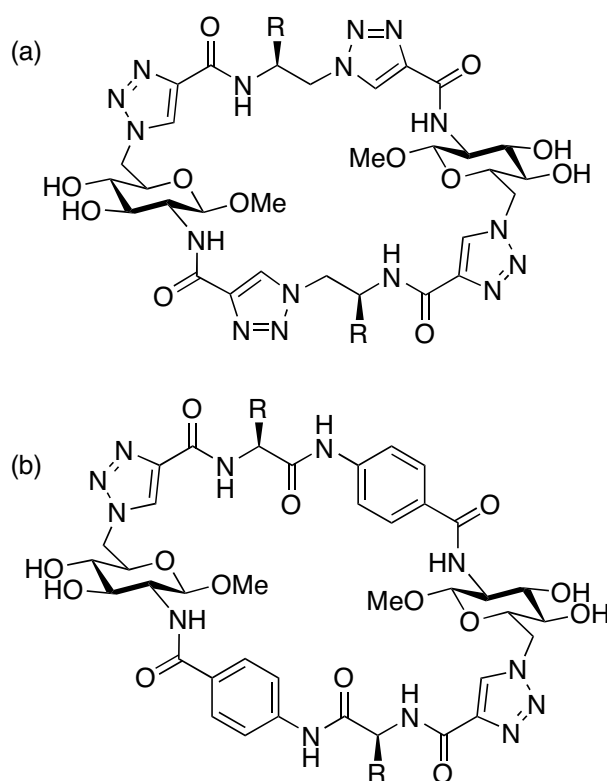
**Figure 8.1** The four possible combinations with tyrosine and arginine as  $\alpha$ -amino acids in macrocycles with three sugar amino acids alternating with three  $\alpha$ -amino acids.

It would also be interesting to prepare macrocycles with four sugar amino acids alternating with four amino acids. Calculations indicate that such molecules would form square structures with sugar amino acids as the sides and amino acids as the corners (Figure 8.2). The relatively large cavity that is formed in the centre of the molecule appears to be a promising binding site for ligands.



**Figure 8.2** Global minimum for a macrocycle with four  $\delta$ -sugar amino acid residues, two tyrosine residues and two arginine residues (MacroModel 8.5, MMFFs force field in water, 20,000 steps, all backbone torsions were selected for random variation).

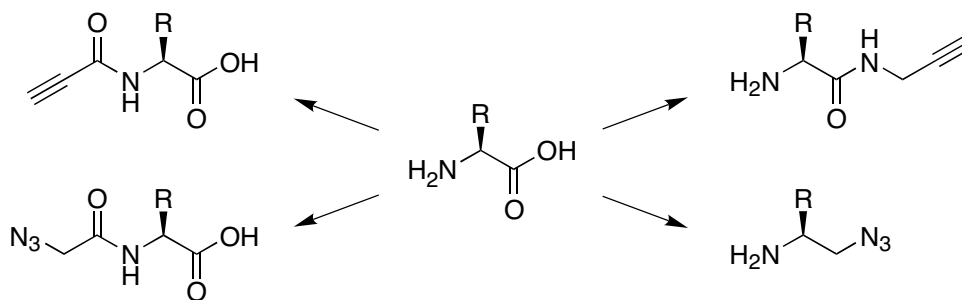
Additionally, the strategy to rigidify the molecules by incorporating aromatic elements such as the 1,2,3-triazoles could be pursued further. Two more amino acids could be replaced with 1,2,3-triazoles and it would also be possible to incorporate other aromatic building blocks, such as *p*-aminobenzoic acid, in the macrocycles (Figure 8.3).



**Figure 8.3** Proposed new types of macrocycles. (a) Replacement of two more tyrosine residues with 1,2,3-triazoles. (b) Replacement of two tyrosine residues with *p*-aminobenzoic acid residues.

Finally, there are many other possibilities to use the formation of 1,2,3-triazoles from azides and terminal alkynes to prepare macrocycles. Suitable building blocks containing azido groups or terminal alkynes can be obtained from amino acids in several ways (Figure 8.4), and many of the methods could also be used with sugar amino acids. Azido groups and terminal alkynes can also be attached to carbohydrates in other ways, and the combinations of the possible building blocks are virtually endless. Macrocycles can either be formed in cyclodimerization reactions as in the work presented in this thesis or in reactions where one component carries two azido groups and the other component carries two terminal alkynes.





**Figure 8.4** Some of the possibilities to prepare building blocks containing azido groups or terminal alkynes from amino acids.

There are thus many possibilities to extend the developed methods to prepare further types of macrocycles for evaluation as artificial receptors. Although only weak interactions were found with the macrocycles described in this thesis, it still seems reasonable that macrocyclic carbohydrate/amino acid hybrids can be efficient artificial receptors given the success of some of the receptors discussed in Chapter 2, in particular the cyclodextrins, the cyclophane by Mallouk and co-workers,<sup>41</sup> and the cyclic peptide by Benito and Meldal.<sup>44</sup> Further research on macrocyclic carbohydrate/amino acid hybrid molecules as artificial receptors is thus warranted.

# 9

## Acknowledgements

---

Under de fem år jag har tillbringat på Organisk kemi 2/Bioorganisk kemi/Organisk kemi så är det många som har hjälpt mig i stort och i smått och allmänt hjälpt till att göra tillvaron på avdelningen trevligare. Jag vill här passa på att tacka dem som jag speciellt kommer ihåg.

- Ulf Nilsson för att ha gett mig den fantastiska möjligheten att genomföra det här projektet samt för allt stöd och all uppmuntran. Stort tack!
- Hans Grundberg, Jörgen Toftered, Pernilla Sörme, Christopher Öberg, Ian Cumpstey, Chris McKay, Kambiz Katebzadeh och Jenny Ågren för trevligt sällskap på labb och skrivrum. Gäller självklart även mina rumsgrannar Karolina Larsson, Olle Hidestål och Johan Tejler. Speciellt tack till Jörgen för otaliga diskussioner kring mer eller mindre lyckade idéer och försök samt för resesällskapet!
- Alla på avdelningen från avdelningsföreståndare Olov Sterner och nedåt för en kreativ miljö och en fantastisk psykosocial arbetsmiljö.
- Anders Sundin för assistans med datorberäkningarna och givande diskussioner kring tolkningen av resultaten.
- Karl-Erik Bergquist och Einar Nilsson för all hjälp med NMR och masspektrometri, jag är medveten om att mina föreningar inte alltid har varit lättanalyserade. Extra tack till Einar för hans hjälp när MALDI:n har trilskats och det har varit dags att gå loss med skruvmejsel och skiftnyckel.
- Ulf Nilsson, Maria Levin och Ulf Lindström för korrekturläsning och givande kommentarer. Tack till Maria även för all hjälp med det byråkratiska och det praktiska under min tid på avdelningen.

- Kursledare Ulf Ellervik och kursdeltagare Magnus Berglund, Jörgen Toftered, Hans Grundberg, Daniel Röme, Johan Eriksson Bajtner och Andréas Meijer för att ha gjort kursen i fysikalorganisk kemi till den succé den var. Värre än Jerry Springer, och samtidigt den bästa kurs jag någonsin gått!
- Kungliga Fysiografiska Sällskapet i Lund för att ha finansierat mitt deltagande i den 39:e IUPAC-konferensen i Ottawa 2003.
- Dessutom vill jag såklart tacka min familj för allt stöd. Jag har inte alltid kunnat förklara så bra vad det egentligen är som jag har gjort, men nu när ni har kunnat läsa avhandlingen så utgår jag från att allt är kristallklart!

# 10

## Supplementary Data

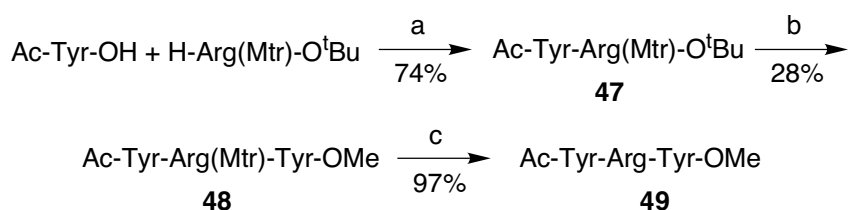
---

General procedures can be found in papers I and III.

### 10.1 Preparation of HAPyU<sup>134,135</sup>

KOAt<sup>74</sup> (292 mg, 1.67 mmol) was suspended in CH<sub>3</sub>CN (9.5 mL, dried over 3 Å MS). Chloro-*N,N,N',N'*-bis(tetramethylene)-formamidinium hexafluorophosphate (557 mg, 1.67 mmol) was added and the mixture was stirred overnight. The insoluble material was filtered off and diethyl ether was added until a precipitate was formed. The mixture was allowed to stand at -20°C for 1 h. The precipitate was filtered off and recrystallized in acetone/diethyl ether to give HAPyU (348 mg, 48%). mp 127-129°C, lit. 118-120°C.<sup>96</sup> The <sup>1</sup>H NMR spectrum was in agreement with published data.<sup>96</sup>

### 10.2 Synthesis of reference tripeptide Ac-Tyr-Arg-Tyr-OMe



**Scheme 10.1** Synthesis of Ac-Tyr-Arg-Tyr-OMe. Reaction conditions: (a) EDC•HCl, HOBT, *N*-methylmorpholine, THF, 16 h; (b) <sup>1</sup>TFA, Et<sub>3</sub>SiH, CH<sub>2</sub>Cl<sub>2</sub>, 4 h; <sup>2</sup>H-Tyr-OMe, EDC•HCl, HOBT, *N*-methylmorpholine, THF, 16 h; (c) TFA/PhSMe, 24 h.

**Ac-Tyr-Arg(Mtr)-O<sup>t</sup>Bu (47).** Ac-Tyr-OH (50.4 mg, 0.226 mmol) was dissolved in THF (2 mL) and H-Arg(Mtr)-O<sup>t</sup>Bu (100 mg, 0.226 mmol), HOBT (30.5 mg, 0.226 mmol), EDC•HCl (45.5 mg, 0.237 mmol) and *N*-methylmorpholine (50 μL, 0.452 mmol) were added. The mixture was stirred overnight and then concentrated. The

product was purified with flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH 15:1, R<sub>f</sub>=0.17) followed by size-exclusion chromatography on a Sephadex LH-20 column (CH<sub>2</sub>Cl<sub>2</sub>:MeOH 1:1) to give **47** (109 mg, 74%). [ $\alpha$ ]<sub>D</sub><sup>22</sup>=+10 (*c* 0.5, MeOH); <sup>1</sup>H NMR (MeOH-d<sub>4</sub>, 400 MHz)  $\delta$  7.05 (d, *J*=8.5 Hz, 2H, Tyr-H <sup>$\delta$</sup> ), 6.68 (d, *J*=8.5 Hz, 2H, Tyr-H <sup>$\epsilon$</sup> ), 6.65 (s, 1H, Mtr-ArH), 4.53 (dd, *J*=9.0 Hz, *J*=5.5 Hz, 1H, Tyr-H <sup>$\alpha$</sup> ), 4.21 (dd, *J*=8.0 Hz, *J*=5.0 Hz, 1H, Arg-H <sup>$\alpha$</sup> ), 3.82 (s, 3H, Mtr-OMe), 3.64 (s, 3H, Tyr-OMe), 3.14 (br m, 2H, Arg-H <sup>$\delta$</sup> ), 3.01 (dd, *J*=14.0 Hz, *J*=5.5 Hz, 1H, Tyr-H <sup>$\beta$</sup> ), 2.75 (dd, *J*=14.0 Hz, *J*=9.1 Hz, 1H, Tyr-H <sup>$\beta$</sup> ), 2.66 (s, 3H, Mtr-Me), 2.60 (s, 3H, Mtr-Me), 2.12 (s, 3H, Mtr-Me), 1.89 (s, 3H, NAc), 1.77 (m, 1H, Arg-H <sup>$\beta$</sup> ), 1.61 (m, 1H, Arg-H <sup>$\beta$</sup> ), 1.50 (m, 2H, Arg-H <sup>$\gamma$</sup> ), 1.42 (s, 9H, O<sup>t</sup>Bu); HRMS (FAB) calcd. for C<sub>31</sub>H<sub>46</sub>N<sub>5</sub>O<sub>8</sub>S (M+H): 648.3067; found 648.3064.

**Ac-Tyr-Arg(Mtr)-Tyr-OMe (48).** Compound **47** (78.2 mg, 0.121 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1.86 mL) and Et<sub>3</sub>SiH (48  $\mu$ L, 0.30 mmol) and TFA (0.93 mL) were added. The mixture was stirred for 4 h and coevaporated with toluene. The crude free acid was dissolved in THF (1 mL) and H-Tyr-OMe (28.0 mg, 0.121 mmol), HOBT (16.3 mg, 0.121 mmol), EDC•HCl (24.3 mg, 0.127 mmol) and *N*-methylmorpholine (40  $\mu$ L, 0.36 mmol) were added. DMSO (0.35 mL) was added and a clear solution was obtained. The mixture was stirred overnight and then concentrated. The product was purified with flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH 9:1, R<sub>f</sub>=0.33) followed by size-exclusion chromatography on a Sephadex LH-20 column (CH<sub>2</sub>Cl<sub>2</sub>:MeOH 1:1). The product was lyophilized from AcOH to give **48** (25.8 mg, 28%). [ $\alpha$ ]<sub>D</sub><sup>22</sup>=+6 (*c* 0.5, MeOH); <sup>1</sup>H NMR (MeOH-d<sub>4</sub>, 400 MHz)  $\delta$  7.02 (d, *J*=8.2 Hz, 2H, Tyr-H <sup>$\delta$</sup> ), 6.98 (d, *J*=8.2 Hz, 2H, Tyr-H <sup>$\delta$</sup> ), 6.69 (d, *J*=8.5 Hz, 2H, Tyr-H <sup>$\epsilon$</sup> ), 6.66 (d, *J*=8.9 Hz, 2H, Tyr-H <sup>$\epsilon$</sup> ), 6.65 (s, 1H, Mtr-ArH), 4.54 (dd, *J*=7.6 Hz, *J*=6.4 Hz, 1H, Tyr-H <sup>$\alpha$</sup> ), 4.47 (dd, *J*=9.0 Hz, *J*=5.6 Hz, 1H, Tyr-H <sup>$\alpha$</sup> ), 4.32 (dd, *J*=8.3 Hz, *J*=5.5 Hz, 1H, Arg-H <sup>$\alpha$</sup> ), 3.82 (s, 3H, Mtr-OMe), 3.64 (s, 3H, Tyr-OMe), 3.13 (br t, *J*=6.5 Hz, 2H, Arg-H <sup>$\delta$</sup> ), 3.05-2.85 (m, 3H, Tyr-H <sup>$\beta$</sup> ), 2.74 (dd, *J*=14.0 Hz, *J*=9.1 Hz, 1H, Tyr-H <sup>$\beta$</sup> ), 2.66 (s, 3H, Mtr-Me), 2.60 (s, 3H, Mtr-Me), 2.12 (s, 3H, Mtr-Me), 1.89 (s, 3H, NAc), 1.72 (m, 1H, Arg-H <sup>$\beta$</sup> ), 1.56 (m, 1H, Arg-H <sup>$\beta$</sup> ), 1.28 (m, 2H, Arg-H <sup>$\gamma$</sup> ); HRMS (FAB) calcd. for C<sub>37</sub>H<sub>49</sub>N<sub>6</sub>O<sub>10</sub>S (M+H): 769.3231; found 769.3252.

**Ac-Tyr-Arg-Tyr-OMe (49).** Compound **48** (17.6 mg, 22.9  $\mu$ mol) was dissolved in TFA containing 5% thioanisole (4 mL). After 24 h, toluene (2 mL) was added and the mixture was concentrated. The product was purified using preparative HPLC (C<sub>18</sub> column, 10 $\rightarrow$ 20% B in A over 30 min, A: H<sub>2</sub>O + 0.1% TFA, B: CH<sub>3</sub>CN + 0.1% TFA, *t*<sub>R</sub> = 11 min) to afford **49** (12.3 mg, 97%). [ $\alpha$ ]<sub>D</sub><sup>22</sup>=+10 (*c* 0.5, MeOH); <sup>1</sup>H NMR (D<sub>2</sub>O,

400 MHz)  $\delta$  7.03 (d,  $J=8.5$  Hz, 2H, Tyr-H<sup>d</sup>), 6.93 (d,  $J=8.5$  Hz, 2H, Tyr-H<sup>d</sup>), 6.74 (d,  $J=8.5$  Hz, 2H, Tyr-H<sup>e</sup>), 6.67 (d,  $J=8.5$  Hz, 2H, Tyr-H<sup>e</sup>), 4.49 (dd,  $J=9.0$  Hz,  $J=5.6$  Hz, 1H, Tyr-H<sup>a</sup>), 4.31 (t,  $J=7.6$  Hz, 1H, Tyr-H<sup>a</sup>), 4.14 (dd,  $J=8.1$  Hz,  $J=6.2$  Hz, 1H, Arg-H<sup>a</sup>), 3.62 (s, 3H, Tyr-OMe), 3.03 (m, 3H, Arg-H<sup>d</sup>+1×Tyr-H<sup>b</sup>), 2.78 (m, 3H, Tyr-H<sup>b</sup>), 1.86 (s, 3H, NAc), 1.55 (m, 1H, Arg-H<sup>b</sup>), 1.47 (m, 1H, Arg-H<sup>b</sup>), 1.34 (m, 2H, Arg-H<sup>c</sup>); HRMS (FAB) calcd. for C<sub>27</sub>H<sub>37</sub>N<sub>6</sub>O<sub>7</sub> (M+H): 557.2724; found 557.2721.

### 10.3 Synthesis of 35

**Methyl 6-azido-3,4-di-O-benzoyl-2,6-dideoxy-2-(9-fluorenylmethoxycarbonyl)-amino- $\beta$ -D-glucopyranoside (35).** *Method A:*<sup>125,126</sup> DEAD (50  $\mu$ L, 0.32 mmol) and DPPA (69  $\mu$ L, 0.32 mmol) were dissolved in THF and the solution was cooled to 0°C. Compound 7 (200 mg, 0.32 mmol) and PPh<sub>3</sub> (84 mg, 0.32 mmol) were added and the mixture was stirred for 30 min at 0°C and 1 h at room temperature. Additional DEAD (100  $\mu$ L, 0.64 mmol), DPPA (140  $\mu$ L, 0.64 mmol) and PPh<sub>3</sub> (168 mg, 0.64 mmol) were added and the solution was stirred for another 1 h. The reaction mixture was concentrated and the product was purified using flash chromatography (Toluene:EtOAc 10:1, R<sub>f</sub>=0.15) to afford **35** (113 mg, 54%). *Method B:*<sup>127</sup> PPh<sub>3</sub> (84 mg, 0.32 mmol) was dissolved in THF and the solution was cooled to 0°C and placed under N<sub>2</sub>. DEAD (50  $\mu$ L, 0.32 mmol) and HN<sub>3</sub> in benzene<sup>128</sup> (160 mM HN<sub>3</sub>, 2 mL, 0.32 mmol) were added dropwise followed by addition of compound 7 (200 mg, 0.32 mmol) and the mixture was stirred at room temperature under N<sub>2</sub>. Additional PPh<sub>3</sub>, DEAD and HN<sub>3</sub> in benzene<sup>128</sup> were added after 15 h, 24 h and 33 h (0.32 mmol of each at each time). After 37 h total reaction time, the reaction mixture was concentrated and the product was purified using flash chromatography (Toluene:EtOAc 8:1, R<sub>f</sub>=0.29) to afford **35** (194 mg, 93%). A fluffy white solid was obtained after lyophilization from benzene.  $[\alpha]_{\text{D}}^{21} = -31$  ( $c$  0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz)  $\delta$  7.83 (m, 4H, 2×Fmoc-H+2×Bz-*o*), 7.75 (d,  $J=7.5$  Hz, 2H, Bz-*o*), 7.63 (m, 2H, NH+1×Bz-*p*), 7.55-7.25 (m, 9H, 4×FmocH+4×Bz-*m*+1×Bz-*p*), 7.14 (q,  $J=8.1$  Hz, 2H, Fmoc), 5.53 (t,  $J=10.0$  Hz, 1H, H-3), 5.22 (t,  $J=9.5$  Hz, 1H, H-4), 4.72 (d,  $J=8.4$  Hz, 1H, H-1), 4.25-3.98 (m, 4H, H-5+3×FmocH), 3.76 (q,  $J=9.5$  Hz, 1H, H-2), 3.60-3.40 (m, 2H, H-6), 3.45 (s, 3H, OMe); HRMS (ESI) calcd. for C<sub>36</sub>H<sub>33</sub>N<sub>4</sub>O<sub>8</sub> (M+H): 649.2298; found 649.2321.

## 10.4 Synthesis of *N*-propiolyl-L-tyrosine *tert*-butyl ester **40**

***N*-Propiolyl-L-tyrosine *tert*-butyl ester (40).** L-Tyrosine *tert*-butyl ester (300 mg, 1.26 mmol) and propiolic acid (78  $\mu$ L, 1.26 mmol) were dissolved in freshly distilled DMF (5 mL) and EEDQ was added (313 mg, 1.26 mmol). The solution was stirred for 16 h and then concentrated. The product was purified using flash chromatography (Toluene:EtOAc 2:1,  $R_f=0.37$ ) to give **40** (280 mg, 77%) as a sticky colourless solid.  $[\alpha]_D^{21}=+82$  ( $c$  0.5,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{MeOH-d}_4$ , 300 MHz)  $\delta$  6.96 (d,  $J=8.5$  Hz, 2H, Tyr-H<sup>d</sup>), 6.64 (d,  $J=8.5$  Hz, 2H, Tyr-H<sup>e</sup>), 4.40 (dd,  $J=8.4$  Hz,  $J=6.6$  Hz, 1H, Tyr-H<sup>a</sup>), 3.52 (s, 1H, CH), 2.92 (dd,  $J=13.9$  Hz,  $J=6.6$  Hz, 1H, Tyr-H<sup>f</sup>), 2.79 (dd,  $J=13.9$  Hz,  $J=8.5$  Hz, 1H, Tyr-H<sup>b</sup>), 1.33 (s, 9H, O<sup>t</sup>Bu); HRMS (ESI) calcd. for  $\text{C}_{12}\text{H}_{10}\text{NO}_3\text{Na}$  ( $\text{M}+\text{Na}$ ): 312.1212; found 312.1203.

# 11

## References

---

1. Stryer, L. *Biochemistry* 4<sup>th</sup> Ed.; W. H. Freeman and Company: New York, 1995.
2. Humphries, M. J.; Newham, P. *Trends Cell Biol.* **1998**, *8*, 78-83.
3. For a general review, see (a) Hartley, J. H.; James, T. D.; Ward, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155-3184. For a review of artificial receptors for carbohydrates, see (b) Davis, A. P.; Wareham, R. S. *Angew. Chem. Int. Ed.* **1999**, *38*, 2978-2996.
4. For examples, see Tobey, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 10963-10970 and references cited therein.
5. For reviews, see (a) Li, S.; Purdy, W. C. *Chem. Rev.* **1992**, *92*, 1457-1470; (b) Snopek, J.; Smolková-Keulemansová, E.; Cserhádi, T.; Gahm, K. H.; Stalcup, A. In *Comprehensive Supramolecular Chemistry*, vol. 3, Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D.; Vögtle, F.; Lehn, J.-M.; Szejtli, J.; Osa, T., Eds.; Pergamon: Oxford, 1996, pp 515-571.
6. Ludwig, R. *Fresen. J. Anal. Chem.* **2000**, *367*, 103-128.
7. Bell, T. W.; Hext, N. M. *Chem. Soc. Rev.* **2004**, *33*, 589-598.
8. Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419-4476.
9. Pietraszkiewicz, M. In *Comprehensive Supramolecular Chemistry*, vol. 10, Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D.; Vögtle, F.; Lehn, J.-M.; Reinhoudt, D. N., Eds.; Pergamon: Oxford, 1996, pp 225-266.
10. *Handbook of Chemical and Biological Sensors*, Taylor, R. F.; Schultz, J. S., Eds.; Institute of Physics Publishing: Bristol, 1996.
11. Han, M. S.; Kim, D. H. *Angew. Chem. Int. Ed.* **2002**, *41*, 3809-3811.



12. Fabbri, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *Angew. Chem. Int. Ed.* **2002**, *41*, 3811-3814.
13. Lavigne, J. J.; Anslyn, E. V. *Angew. Chem. Int. Ed.* **1999**, *38*, 3666-3669.
14. Piatek, A. M.; Bomble, Y. J.; Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 6072-6077.
15. Metzger, A.; Anslyn, E. V. *Angew. Chem. Int. Ed.* **1998**, *37*, 649-652.
16. Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 10109-10110.
17. Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423-428.
18. Schmuck, C. *Chem. unserer Zeit*, **2001**, *35*, 356-366.
19. Casnati, A.; Fabbi, M.; Pelizzi, N.; Pochini, A.; Sansone, F.; Ungaro, R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2699-2704.
20. Xu, R.; Greiveldinger, G.; Marenus, L. E.; Cooper, A.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 4898-4899.
21. Dong, D. L.; Liu, R.; Sherlock, R.; Wigler, M. H.; Nestler, H. P. *Chem. Biol.* **1999**, *6*, 133-141.
22. Cameron, K. S.; Fielding, L.; Mason, R.; Muir, A. W.; Rees, D. C.; Thorn, S.; Zhang, M.-Q. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 753-755.
23. Bom, A.; Bradley, M.; Cameron, K.; Clark, J. K.; van Egmond, J.; Feilden, H.; MacLean, E. J.; Muir, A. W.; Palin, R.; Rees, D. C.; Zhang, M.-Q. *Angew. Chem. Int. Ed.* **2002**, *41*, 265-270.
24. Creighton, T. E. *Proteins—Structures and Molecular Properties 2<sup>nd</sup> Ed.*; W. H. Freeman and Company: New York, 1993.
25. For recent reviews of receptors that form boronate esters with vicinal diols, see (a) Striegler, S. *Curr. Org. Chem.* **2003**, *7*, 81-102; (b) James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159-200.
26. Schneider, H.-J.; Mohammad-Ali, A. K. In *Comprehensive Supramolecular Chemistry*, vol. 2, Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D.; Vögtle, F.; Lehn, J.-M., Eds.; Pergamon: Oxford, 1996, pp 69-101.
27. Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem. Int. Ed.* **2003**, *42*, 1210-1250.
28. Cram, D. J. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 1039-1057.

29. Koshland, D. E., Jr. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2375-2378.
30. Fenyvesi, E.; Szente, L.; Russell, N. R.; McNamara, M. In *Comprehensive Supramolecular Chemistry*, vol. 3, Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D.; Vögtle, F.; Szejtli, J.; Osa, T., Eds.; Pergamon: Oxford, 1996, pp 305-366.
31. Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743-1753.
32. Benito, J. M.; Jiménez Blanco, J. L.; Mellet, C. O.; García Fernández, J. M. *Angew. Chem. Int. Ed.* **2002**, *41*, 3674-3676.
33. Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. *J. Am. Chem. Soc.* **2001**, *123*, 8189-8196.
34. Dondoni, A.; Marra, A.; Scherrmann, M.-C.; Casnati, A.; Sansone, F.; Ungaro, R. *Chem. Eur. J.* **1997**, *3*, 1774-1782.
35. (a) Coterón, J. M.; Vicent, C.; Bosso, C.; Penadés, S. *J. Am. Chem. Soc.* **1993**, *115*, 10066-10076. (b) Jiménez-Barbero, J.; Junquera, E.; Martín-Pastor, M.; Sharma, S.; Vicent, C.; Penadés, S. *J. Am. Chem. Soc.* **1995**, *117*, 11198-11204.
36. Srinivasan, N.; Kilburn, J. D. *Curr. Opin. Chem. Biol.* **2004**, *8*, 305-310.
37. Billing, J.; Grundberg, H.; Nilsson, U. J. *Supramol. Chem.* **2002**, *14*, 367-372.
38. Grundberg, H. Doctoral thesis, Lund University, 2004.
39. Brewster, R. E.; Caran, K. L.; Sasine, J. S.; Shuker, S. B. *Curr. Org. Chem.* **2004**, *8*, 867-881.
40. Sansone, F.; Barbosa, S.; Casnati, A.; Sciotto, D.; Ungaro, R. *Tetrahedron Lett.* **1999**, *40*, 4741-4744.
41. Gavin, J. A.; Garcia, M. E.; Benesi, A. J.; Mallouk, T. E. *J. Org. Chem.* **1998**, *63*, 7663-7669.
42. Jensen, K. B.; Braxmeier, T. M.; Demarcus, M.; Frey, J. G.; Kilburn, J. D. *Chem. Eur. J.* **2002**, *8*, 1300-1309.
43. Sugimoto, N.; Miyoshi, D.; Zou, J. *Chem. Commun.* **2000**, 2295-2296.
44. Benito, J. M.; Meldal, M. *QSAR Comb. Sci.* **2004**, *23*, 117-129.
45. Kubik, S.; Goddard, R.; Kirchner, R.; Nolting, D.; Seidel, J. *Angew. Chem. Int. Ed.* **2001**, *40*, 2648-2651.

46. Kubik, S.; Kirchner, R.; Nolting, D.; Seidel, J. *J. Am. Chem. Soc.* **2002**, *124*, 12752-12760.
47. Otto, S.; Kubik, S. *J. Am. Chem. Soc.* **2003**, *125*, 7804-7805.
48. Bitta, J.; Kubik, S. *Org. Lett.* **2001**, *3*, 2637-2640.
49. Kubik, S.; Goddard, R. *Eur. J. Org. Chem.* **2001**, 311-322.
50. Sugar amino acids have been reviewed extensively in recent years: (a) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491-514; (b) Schweizer, F. *Angew. Chem. Int. Ed.* **2002**, *41*, 230-253; (c) Chakraborty, T.K.; Ghosh, S.; Jayaprakash, S. *Curr. Med. Chem.* **2002**, *9*, 421-435; (d) Peri, F.; Cipolla, L.; Forni, E.; La Ferla, B.; Nicotra, F. *Chemtracts Org. Chem.* **2001**, *14*, 481-499.
51. Knapp, S. *Chem. Rev.* **1995**, *95*, 1859-1876.
52. Heyns, K.; Paulsen, H. *Chem. Ber.* **1955**, *88*, 188-195.
53. van Well, R. M.; Overkleeft, H. S.; Overhand, M.; Vang Carstenen, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **2000**, *41*, 9331-9335.
54. Mayes, B. A.; Stetz, R. J. E.; Ansell, C. W. G.; Fleet, G. W. J. *Tetrahedron Lett.* **2004**, *45*, 153-156.
55. Mayes, B. A.; Simon, L.; Watkin, D. J.; Ansell, C. W. G.; Fleet, G. W. J. *Tetrahedron Lett.* **2004**, *45*, 157-162.
56. Mayes, B. A.; Cowley, A. R.; Ansell, C. W. G.; Fleet, G. W. J. *Tetrahedron Lett.* **2004**, *45*, 163-166.
57. Sofia, M. J.; Hunter, R.; Chan, T. Y.; Vaughan, A.; Dulina, R.; Wang, H.; Gange, D. *J. Org. Chem.* **1998**, *63*, 2802-2803.
58. Fuchs, E.-F.; Lehmann, J. *Chem. Ber.* **1975**, *108*, 2254-2260.
59. Nicolaou, K. C.; Flörke, H.; Egan, M. G.; Barth, T.; Estevez, V. A. *Tetrahedron Lett.* **1995**, *36*, 1775-1778.
60. Suhara, Y.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575-1578.
61. Timmers, C. M.; Turner, J. J.; Ward, C. M.; van der Marel, G. A.; Kouwijzer, M. L. C. E.; Grootenhuis, P. D. J.; van Boom, J. H. *Chem. Eur. J.* **1997**, *3*, 920-929.
62. Graf von Roedern, E.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 687-689.

63. Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156-10167.
64. Lohof, E.; Planker, E.; Mang, C.; Burkhart, F.; Dechantsreiter, M. A.; Haubner, R.; Wester, H.-J.; Schwaiger, M.; Hölzemann, G.; Goodman, S. L.; Kessler, H. *Angew. Chem. Int. Ed.* **2000**, *39*, 2761-2763.
65. van Well, R. M.; Overkleeft, H. S.; van der Marel, G. A.; Bruss, D.; Thibault, G.; de Groot, P. G.; van Boom, J. H.; Overhand, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 331-334.
66. Gruner, S. A. W.; Kéri, G.; Schwab, R.; Venetianer, A.; Kessler, H. *Org. Lett.* **2001**, *3*, 3723-3725.
67. Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stöckle, M.; Kessler, H. *Chem. Eur. J.* **2002**, *8*, 4365-4376.
68. For a thorough overview of all aspects of peptide chemistry, see *Houben-Weyl Methods of Organic Chemistry 4<sup>th</sup> Ed.*, vols E22a-d, Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C., Eds.; Georg Thieme Verlag: Stuttgart, 2002.
69. For a recent review, see Han, S.-Y.; Kim, Y.-A. *Tetrahedron* **2004**, *60*, 2447-2467.
70. Sheehan, J.C.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067-1068.
71. König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788-798.
72. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397-4398.
73. Albericio F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678-9683.
74. Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem. Int. Ed.* **2002**, *41*, 441-445.
75. Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *16*, 1219-1222.
76. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205-208.
77. Coste, J.; Frérot, E.; Jouin, P. *Tetrahedron Lett.* **1991**, *32*, 1967-1970.
78. Coste, J.; Frérot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437-2446.
79. Belleau, B.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651-1652.

80. Kiso, Y.; Kai, Y.; Yajima, H. *Chem. Pharm. Bull.* **1973**, *21*, 2507-2510.
81. Romoff, T. T. In *Houben-Weyl Methods of Organic Chemistry 4<sup>th</sup> Ed.*, vol. E22b, Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C., Eds.; Georg Thieme Verlag: Stuttgart, 2002, section 7.4.
82. Kemp, D. S. In *The Peptides—Analysis, Synthesis, Biology*, vol. 1, Gross E.; Meienhofer J., Eds.; Academic Press: New York, 1979, pp 315-383.
83. Quibell, M.; Packman, L. C.; Johnson, T. J. *Chem. Soc., Perkin Trans. 1*, **1996**, 1219-1225.
84. Griehl, C.; Weigt, J.; Jeschkeit, H. *J. High Res. Chromatog.* **1994**, *17*, 700-704.
85. Miyazawa, T.; Otomatsu, T.; Fukui, Y.; Yamada, T.; Kuwata, S. *Int. J. Peptide Protein Res.* **1992**, *39*, 237-244.
86. Nishiyama, Y.; Tanaka, M.; Saito, S.; Ishizuka, S.; Mori, T.; Kurita, K. *Chem. Pharm. Bull* **1999**, *47*, 576-578.
87. Irvine, J. C.; McNicoll, D.; Hynd, A. *J. Chem. Soc.* **1911**, *11*, 250-261.
88. Yamasaki, T.; Kubota, Y.; Tsuchiya, T.; Umezawa, S. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3190-3192.
89. Onodera, K.; Kitaoka, S.; Ochiai, H. *J. Org. Chem.* **1962**, *27*, 156-159.
90. Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1993**, *34*, 1181-1184.
91. Ying, L.; Gervay-Hague, J. *Carbohydr. Res.* **2004**, *339*, 367-375.
92. Fugedi, P.; Csaba, P. U.S. Patent 5 849 709, 1998.
93. Mehta, A.; Jaouhari, R.; Benson, T. J.; Douglas, K. T. *Tetrahedron Lett.* **1992**, *33*, 5441-5444.
94. Sheppeck, J. E., II; Kar, H.; Hong, H. *Tetrahedron Lett.* **2000**, *41*, 5329-5333.
95. Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831-8838.
96. Carpino, L. A.; El-Faham, A.; *J. Org. Chem.* **1994**, *59*, 695-698.
97. ElAmin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442-3444.
98. Fujino, M.; Wakimasu, M.; Kitada, C. *Chem. Pharm. Bull.* **1981**, *29*, 2825-2831.
99. Wilcox, C. S. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*, Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, 1990, pp 123-143.
100. Fielding, L. *Tetrahedron* **2000**, *56*, 6151-6170.
101. Lawaczeck, R.; Wagner, K. G. *Biopolymers* **1974**, *13*, 2003-2014.
102. Yamauchi, O.; Odani, A.; Masuda, H.; Sigel, H. In *Metal Ions in Biological Systems* vol. 32, Sigel, A.; Sigel, H., Eds.; Marcel Dekker: New York, 1996, pp 207-270.

103. Aoki, S.; Kimura, E. *Rev. Mol. Biotech.* **2002**, *90*, 129-155.
104. Butterfield, S. M.; Waters, M. L. *J. Am. Chem. Soc.* **2003**, *125*, 9580-9581.
105. Piantanida, I.; Palm, B. S.; Cudic, P.; Zinic, M.; Schneider, H.-J. *Tetrahedron Lett.* **2001**, *42*, 6779-6783.
106. Gao, H.; Cai, L.; Qi, Y.; Wang, H. *Supramol. Chem.* **2003**, *15*, 323-325.
107. Abe, H.; Mawatari, Y.; Teraoka, H.; Fujimoto, K.; Inouye, M. *J. Org. Chem.* **2004**, *69*, 495-504.
108. Ueki, M.; Nishigaki, N.; Aoki, H.; Tsurusaki, T.; Katoh, T.; *Chem. Lett.* **1993**, 721-724.
109. Kartha, K. P. R.; Mukhopadhyay, B.; Field, R. A. *Carbohydr. Res.* **2004**, *339*, 729-732.
110. Akiya, S.; Osawa, T. *Chem. Pharm. Bull.* **1960**, *8*, 583-586.
111. Bundle, D. R.; Josephson, S. *Can. J. Chem.* **1980**, *58*, 2679-2685.
112. Schwartz, D. A.; Lee, H.-H.; Carver, J. P.; Krepinsky, J. J. *Can. J. Chem.* **1985**, *63*, 1073-1079.
113. Grundler, G.; Schmidt, R. R. *Carbohydr. Res.* **1985**, *135*, 203-218.
114. Campos-Valdes, M. T.; Marino-Albernas, J. R.; Verez-Bencomo, V. *J. Carbohydr. Chem.* **1987**, *6*, 509-513.
115. Alais, J.; David, S. *Carbohydr. Res.* **1990**, *201*, 69-77.
116. Zuurmond, H. M.; van der Klein, P. A. M.; de Wildt, J.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1994**, *13*, 323-339.
117. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis 3<sup>rd</sup> Ed*; John Wiley & Sons: New York, 1999, p. 566.
118. Ohtsuka, I.; Hada, N.; Ohtaka, H.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **2002**, *50*, 600-604.
119. Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. *Synthesis* **1994**, 1063-1066.
120. Corey, E. J.; Samuelsson, B. *J. Org. Chem.* **1984**, *49*, 4735-4735.
121. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.
122. Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057-3064.
123. For examples of use in bioorganic chemistry, see Breinbauer, R.; Köhn, M. *ChemBioChem* **2003**, *4*, 1147-1149.
124. Bodine, K. D.; Gin, D. Y.; Gin, M. S. *J. Am. Chem. Soc.* **2004**, *126*, 1638-1639.
125. Lal, B.; Pramanik, B. N.; Manhas, M. S.; Bose, A. K. *Tetrahedron Lett.* **1977**, *18*, 1977-1980.

126. Thompson, A. S.; Humphrey, G. R.; DeMarco, A. M.; Mathre, D. J.; Grabowski, E. J. *J. Org. Chem.* **1993**, *58*, 5886-5888.
127. Boeijen, A.; van Ameijde, J.; Liskamp, R. M. J. *J. Org. Chem.* **2001**, *66*, 8454-8462.
128. Wolff, H. In *Organic Reactions* vol 3, Adams, R.; Bachmann, W. E.; Fieser, L. F.; Johnson, J. R.; Snyder, H. R., Eds.; John Wiley & Sons: New York, 1946, p. 327.
129. Ghosh, S. S.; Dakoji, S.; Tanaka, Y.; Cho, Y. J.; Mobashery, S. *Bioorg. Med. Chem.* **1996**, *4*, 1487-1492.
130. Kunishima, M.; Kawachi, C.; Morita, J.; Terao, K.; Iwasaki, F.; Tani, S. *Tetrahedron* **1999**, *55*, 13159-13170.
131. Birtwistle, D. H.; Brown, J. M.; Foxton, M. W. *Tetrahedron* **1988**, *44*, 7309-7318.
132. Andrus, M. B.; Lepore, S. D.; Turner, T. M. *J. Am. Chem. Soc.* **1997**, *119*, 12159-12169.
133. Sharpless, B. K.; Fokin, V.; Rostovtsev, V.; Green, L.; Himo, F. *PCT Int. Appl.* WO2003101972, 2003.
134. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927-1930.
135. Bienert, M.; Henklein, P.; Beyermann, M.; Carpino, L. A. In *Houben-Weyl Methods of Organic Chemistry 4<sup>th</sup> Ed.*, vol. E22a, Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C., Eds.; Georg Thieme Verlag: Stuttgart, 2002, section 3.8, p. 562.