Synthesis, characterization and crystal ctructure of a novel pyridyl urea macrocycle

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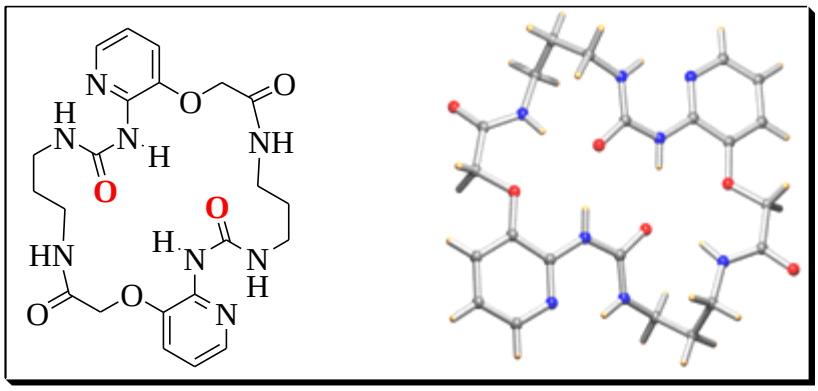
Abstract Graphic:



Abstract: A novel pyridyl urea based macrocycle has been synthesized and fully characterized including a single crystal X-ray structure determination. The synthetic approach first involves the reaction of benzyloxycarbonylaminopropyl-3-isocayanate with *t*-butyl 2-(2-aminopyridin-3-yloxy) acetate resulting in a coupling product. After deprotection of the amine and acid moieties and coupling between these in the presence of EDC, a macrocycle **1** is formed. The structures of the compounds were confirmed by mass and NMR spectroscopy and the X-ray crystal structure of the macrocycle reveals as expected a non-binding conformation with an intramolecular hydrogen bond between the urea N-Hs and the pyridyl-Ns.

Keywords: macrocycle; x-ray crystal structure; intramolecular hydrogen bonding

1. Introduction

Association between host and guest molecules is usually based on simultaneous non-covalent intermolecular interactions between the acceptor and donor, such as a cation and anion, or a

hydrogen-bond acceptor-donor. The non-covalent interactions are generally weak; therefore multiple interactions are important to achieve strong and selective complexation of a guest molecule. Many macrocyclic systems for binding anions have been reported. These systems can incorporate one or more anionic binding group such as thiourea, urea, guanidinium and ammonium. Kilburn and coworkers have synthesised chiral pyridyl-bisthiourea macrocycles and studied their binding properties with various dicarboxylate salts.[1]

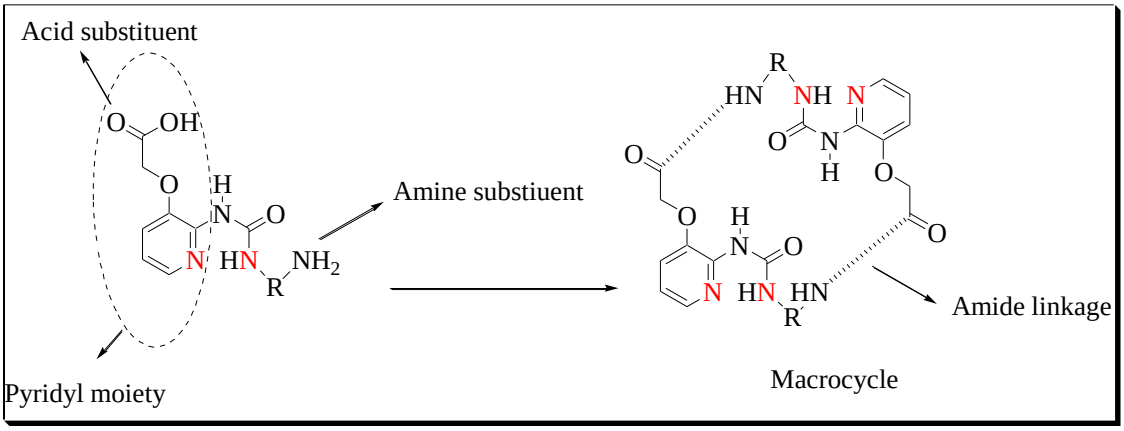


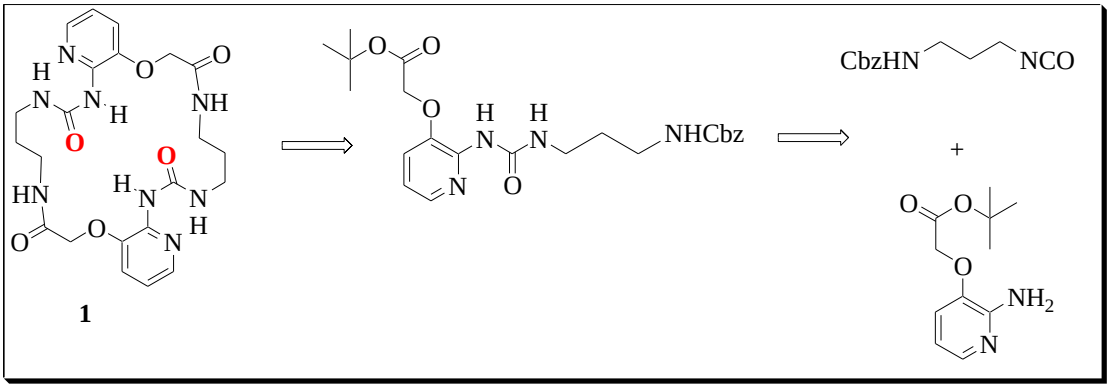
Figure 1. The general binding and non-binding conformations in a *N*-pyridyl urea macrocycle.

The aim of this work was to prepare a cyclic *N*-pyridyl urea receptor which was expected to have a similar non-binding conformation (**X**) to the acyclic analogues. Thus, we anticipated that protonation of these receptors could switch their structural conformation and the binding properties in a similar manner to those of their acyclic counterparts (Figure 1). It was also expected that the anion complexation of these cyclic systems would be stronger than that for their acyclic analogues due to the macrocyclic effect.

2. Results

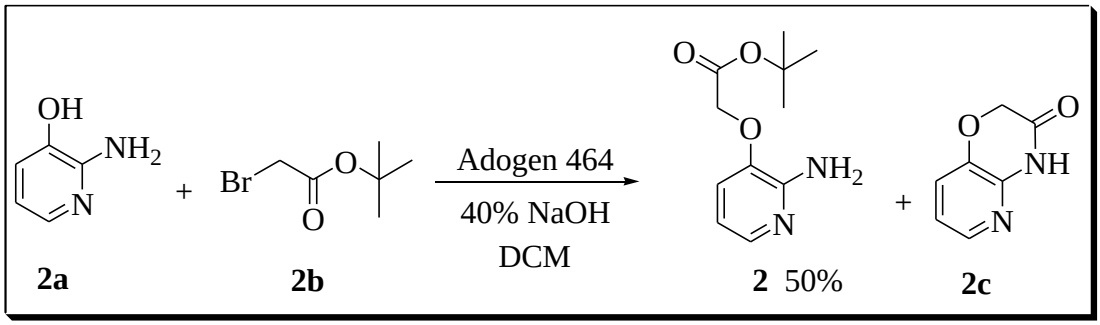
The cyclic *N*-pyridyl urea was synthesized by different reactions and all compounds were purified through column chromatography and their structures were confirmed using standard

instrumental techniques. The attempt to synthesize macrocycle **1** is shown in the retro-synthetic analysis (Scheme 1).



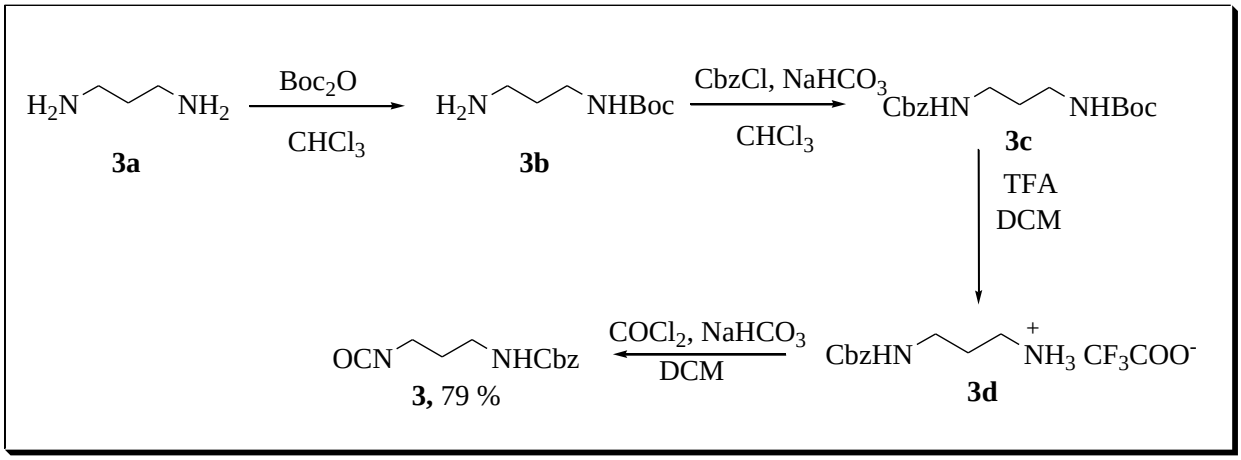
Scheme 1. Retro-synthesis scheme of *N*-pyridyl urea receptor.

N-pyridyl urea macrocycle **1** is composed of two symmetric urea moieties linked together through amide bonds. Amine **2** was used as the pyridyl moiety for the synthesis of compound **1**. Adogen 464 was used as a phase transfer catalyst, which is soluble in the organic phase andis an anionic reagent, which is soluble in the aqueous phase. The catalyst brings the anionic reagent into the organic phase where it reacts with the substrate. The yield of this reaction was generally low (40-50 %) presumably due to the competitive formation of the intramolecular cyclic amide **2c**. (Scheme 2). The acyclic urea molecule containing acid and amine substituents was first prepared by the coupling of isocyanate and amine under neutral reaction condition, followed by the cleavage of the Boc group from **3c** by the formation of the corresponding TFA salt and then its conversion into isocyanate **3**.



Scheme 2: Amine **2** was used as the pyridyl moiety.

The isocyanate **3** was prepared starting from commercially available 1,3-diaminopropane **3a**, which was mono-protected using *tert*-butyldicarbonate giving amine **3b** and di-protected with CbzCl to give the protected compound **3c**, which was then converted into the corresponding isocyanate **3** in the presence of trifluoroacetic acid and phosgene solution (Scheme 3).



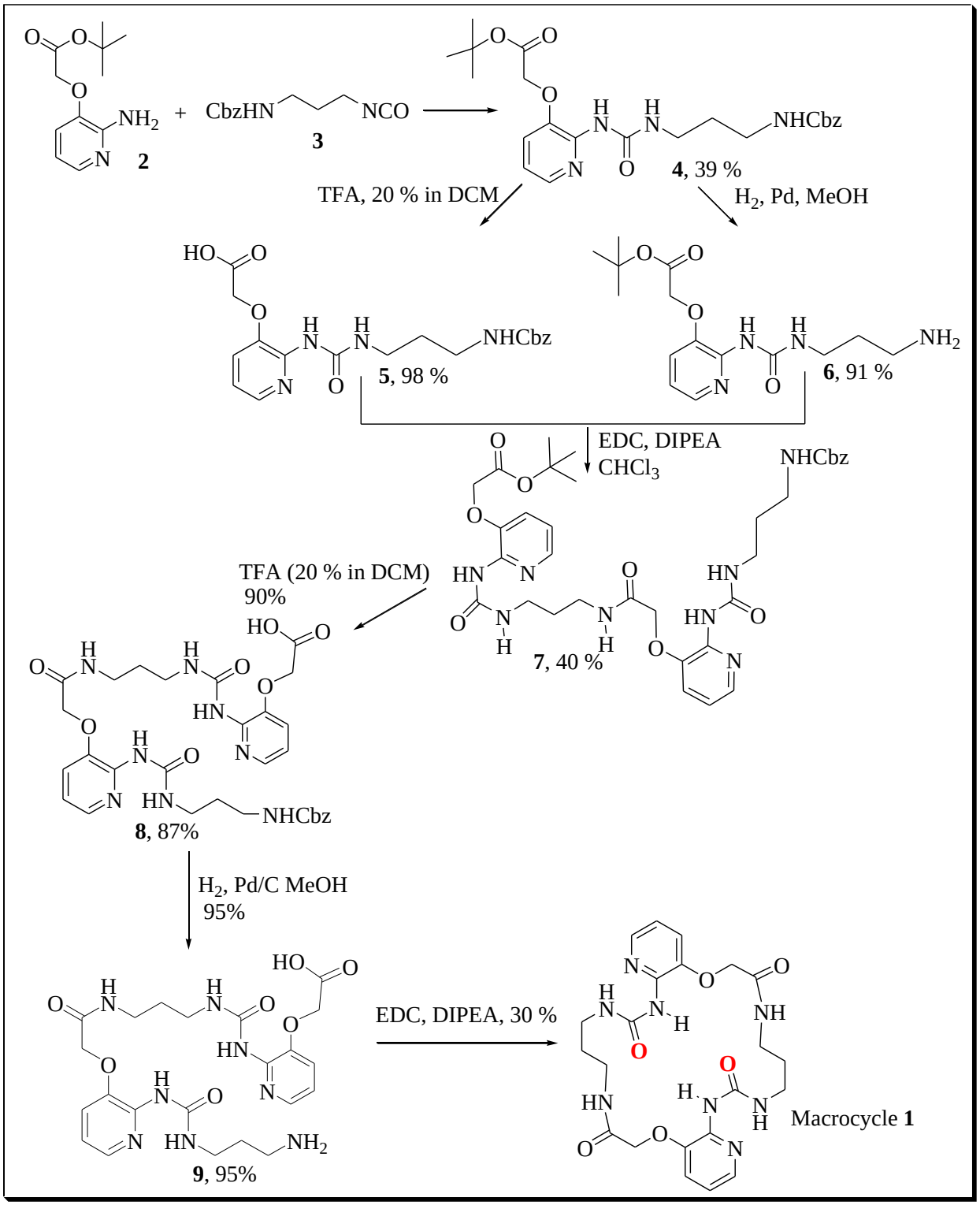
Scheme 3: Synthesis of precursor **3**.

The reaction with triphosgene and diphosgene was straight forward but the yield of the isocyanate **3** was low and the reaction time was long. In contrast, the reaction with phosgene was very sensitive and the reaction took less time for completion with a high yield (Table 1).

Table 1: Conditions used for the synthesis of isocyanate **3**.

Entry	Condition	yield of 3 %
1	Triphosgene, DCM, 1M NaOH, 17, rt	30
2	Diphosgene, DCM, 1M NaOH, 17, rt	27
3	Phosgene, DCM, aq $NaHCO_3$, 10h, 0^oC to rt	79

Coupling of amine **2** with aryl isocyanate **3** gave acyclic urea **4** which is the key intermediate for the synthesis of macrocycle **1** (scheme 4).



Scheme 4: Synthetic route of macrocycle **1**

In the next step, the urea **4** is coupled via amide bonds to form the symmetric macrocycle **1**. Three cyclization methods were used in this study and the highest yield was obtained using EDC and DIPEA as a base and $CHCl_3$ as the solvent at room temperature to give **1** in 30 % yield (Table 2).

Table 2: Cyclization step in different conditions.

Entry	Condition	% of **1**
1	Pybop, DIPEA, $CHCl_3$, rt, 48h	19
2	DDC, DIPEA, $CHCl_3$, rt, 48h	20
3	EDC, DIPEA, $CHCl_3$, rt, 48h	30

3. Discussion

3.1. X–ray structure and NMR analysis of macrocycle 1

The X-ray crystal structure of macrocycle **1** reveals the expected non-binding conformation **X** with an intramolecular hydrogen bond between the urea N-Hs and the pyridyl-Ns, and with N and O, a N_3H …N_1 distance of 2.01 Å, N_4H …O_1 distance of 1.97 Å, a N_2H …O_1 distance of 2.01 Å and a N_4H …O_2 distance of 2.19 Å, while the distances of N…..O and N…N were N3…N1 distance of 2.72, N4…O1 distance of 2.78 Å and N2…O1 distance of 2.85 Å (Figure 2). This conformation was also confirmed by the ^1H NMR spectrum, as it revealed a downfield shift of 10.2 ppm (relative to TMS in $CDCl_3$) for the bonded N_3-H and chemical shifts of 9.58 and 9.88 ppm for the unbound N_2-H and amide N_4-H, respectively.

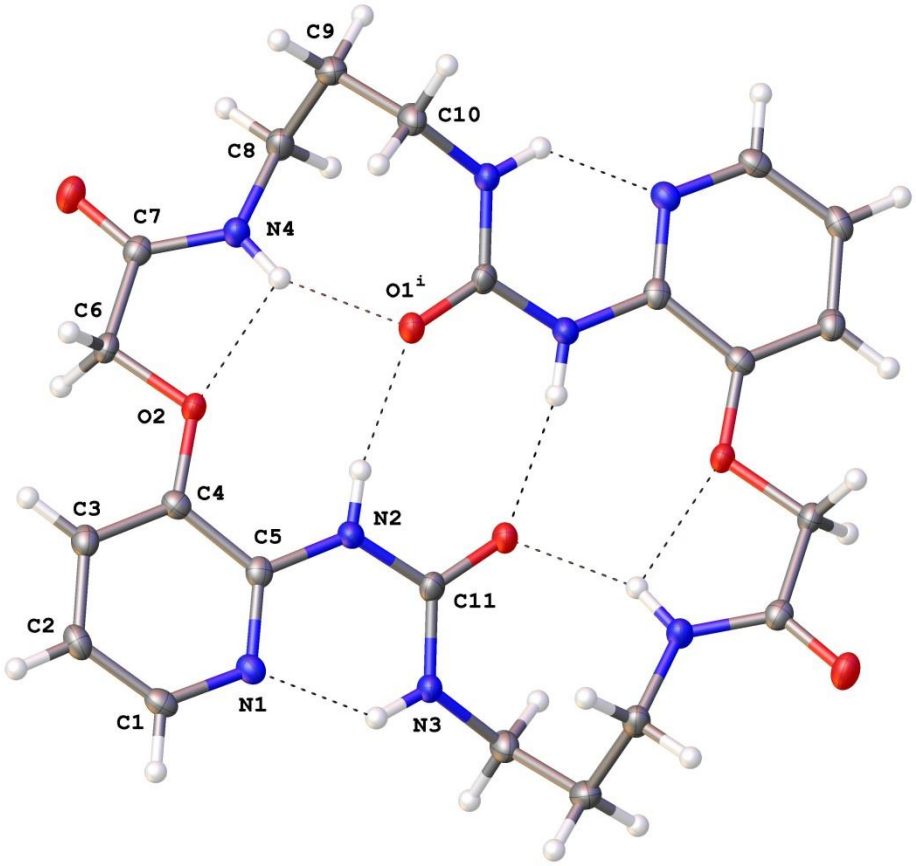


Figure 2. X-ray crystal structure of macrocycle **1** showing labelling scheme and hydrogen bond interactions. Distances are given in full in table 3. Thermal ellipsoids drawn at the 35% probability level.

Macrocycle **1** was sparingly soluble in a range of solvents but only soluble in a 1:1 mixture of chloroform: methanol. Crystals were grown from a (1:1 chloroform: methanol): diethyl ether mixture and the single crystal X-ray structure is shown in Figure 2.

3.2. Attempts to synthesize the protonated salt of macrocycle 1

Treatment of macrocycle **1** with two equivalents of hydrochloric acid, hydrobromic acid, tetrafluoroboric acid and sulphuric acid in 1:1 chloroform: methanol mixture led to precipitates of the corresponding salts. These salts were sparingly soluble in a range of solvents such as CH_3CN, $CHCl_3$ and MeOH and only soluble in DMSO. Attempt at solubilisation in DMSO led to deprotonation of the salt giving back the neutral macrocycle as confirmed by NMR spectroscopy. As a result of this solubility limitation, it would be difficult to follow the NMR spectrum signals for both neutral macrocycle and its corresponding salts in the same solvent.

4. Materials and Methods

4.1. General

This part is given as supplementary material.

4.2. Synthesis and Characterization of *t*-butyl 2-(2-aminopyridin-3-yloxy) acetate 2

A mixture of 3-hydroxy-2-aminopyridine **2a** (3 g, 27 mmol), *tert*-butylbromoacetae **2b** (4 ml, 27 mmol), Adogen-464 (168 mg) and sodium hydroxide solution (40% in water, 15 ml) was dissolved in dichloromethane (25 ml). The reaction mixture was stirred at room temperature for 24 h. The mixture was then washed with water (3 x 50 ml) and the combined organic layer was dried over magnesium sulfate, filtered and concentrated in *vacuo* to leave a crude yellow oil.

This was purified by column chromatography over silica gel using 3 % methanol: dichloromethane as eluent to give amine **2** (2.3 g, 10.3 mmol, 38 %) as a yellow oil. R_f (5% methanol/ dichloromethane) 0.50; *v* (film)/cm^{-1} 3483 (w), 1748 (s), 1616 (s), 1476 (s), 1154 (s); δ_H (300 MHz, $CDCl_3$); 7.67 (1H, d, *J* = 5 Hz, H py), 6.80 (1H, d, *J* = 8 Hz, H py), 6.55 (1H, dd, *J* = 8, 5 Hz, H py), 4.85 (2H, br s, NH_2), 4.49 (2H, s, CH_2), 1.45 (9H, s, $3CH_3$) ppm; δ_C (75 MHz, $CDCl_3$); 167.7 (s), 150.7 (s), 140.9 (s), 140.1 (d), 117.7 (d), 113.3 (d), 82.9 (s), 66.3 (t), 28.2 (q) ppm; LRMS (ES^+) *m/z* (%) 266 $[M + H + CH_3CN]^+$ (30).[2-4]

4.3. Synthesis and characterization of *t*-butyl 2-(3-aminopropylamino) acetate 3b

A solution of *tert*-butyl dicarbonate (9.8 g, 45 mmol) in chloroform (300 ml) was added drop wise to a solution of 1,3-propanediamine (10 g, 135 mmol) in chloroform (30 ml) over 8 h, and the reaction mixture was then stirred at room temperature for 15 h. The reaction mixture was washed with saturated sodium bicarbonate solution (4 x 100 ml) and water (3 x 100 ml) and the organic phase was separated, dried over magnesium sulfate and concentrated in *vacuo* to give amine **3b** (5.41 g, 31 mmol, 69 %) as a colourless oil, which was used without further purification. *v* (film)/ cm^{-1} 3357 (w), 2975 (w), 1689 (s), 1517 (s), 1250 (s); δ_H (300 MHz, $CDCl_3$) 5.15 (1H, br s, *NH*Boc), 3.04 (2H, m, CH_2NHBoc), 2.62 (2H, t, *J* = 7 Hz, CH_2NH_2), 1.47 (2H, quint, *J* = 7 Hz, $CH_2CH_2CH_2$), 1.31 (9H, s, $3CH_3$) 1.28 (2H, br s, NH_2) ppm; δ_C (75 MHz, $CDCl_3$) 155.4 (s), 77.8 (s), 38.6 (t), 37.3 (t), 32.5 (t), 26.9 (q) ppm; LRMS (ES^+) *m/z* (%) 175 $[M + H]^+$ (100), 215 $[M + CH_3CN]^+$ (25). The data coincided with those reported in literature.[5]

4.4. Synthesis and characterization of *t*-butyl 2-(3-benzyloxycarbonylaminopropylamino) acetate 3c

Benzyl chloroformate CbzCl (7.3 ml, 51.1 mmol) was added to a solution of amine **3b** (8.9 g, 51 mmol) in chloroform (150 ml) and saturated sodium bicarbonate (30 mL). The mixture was stirred at room temperature for 12 h and then washed with saturated sodium bicarbonate solution (2 x 100 ml) and the aqueous layer was washed with chloroform (3 x 50 ml). The combined organic phases were dried over magnesium sulfate, filtered and concentrated in *vacuo* to give carbamate **3c** (12.70 g, 41.2 mmol, 81 %) as a white solid, which was used without further purification. ν (film)/ cm^{-1} 3334 (m), 2977 (w), 1683 (s), 1528 (s), 1365 (w), 1249 (s), 1166 (s); δ_H (300 MHz, $CDCl_3$) 7.18 (5H, m, 5H Ar), 5.41 (1H, br s, *NH*Cbz), 5.16 (2H, s, CH_2Ph), 4.75 (1H, br s, *NH*Boc), 3.12-3.02 (4H, m, $2CH_2$), 1.47 (2H, quint, J = 6 Hz, $CH_2CH_2CH_2$), 1.30 (9H, s, $3CH_3$) ppm; δ_C (75 MHz, $CDCl_3$) 156.7 (s), 156.4 (s), 136.6 (s), 129.4 (s), 128.9 (s), 127.0 (s), 79.4 (s), 73.4 (s), 37.8 (s), 37.5 (s), 30.6 (s), 28.4 (s) ppm; LRMS (ES^+) *m/z* (%) 309 $[M + H]^+$ (12), 331 $[M + Na]^+$ (100), 372 $[M + Na + CH_3CN]^+$ (13), 639 $[2 M + Na]^+$ (15).[6]

4.5. 3-Benzyloxycarbonylaminopropylammonium trifluoroacetate 3d

A solution of **3c** (11.00 g, 35.60 mmol) in trifluoroacetic acid, TFA, (20 % in dichloromethane, 50 mL) was stirred at room temperature for 2 hr. Toluene (50 mL) was added and the solvent was removed under reduced pressure to give salt **3d** (9.70 g, 30.00 mmol, 84 %) as a white solid, which was used without further purification. ν (film)/ cm^{-1} 2435 (w), 2962 (w), 2689 (s), 1668 (m), 1524 (s), 1131 (s); δ_H (300 MHz, $CDCl_3$) 8.00 (3H, br s, N^+H_3), 7.18 (5H, m, 5H Ar), 5.45 (1H, br s, *NH*Cbz), 4.96 (2H, br s, CH_2Ph), 3.03 (2H, br s, $CH_2N^+H_3$), 2.79 (2H, br s, CH_2NHCbz), 1.62 (2H, quint, J = 6 Hz, $CH_2CH_2CH_2$) ppm; δ_C (75 MHz, $CDCl_3$) 161.3 (s), 136.6 (s), 129.4 (d), 128.7 (d), 127.5 (d), 67.4 (t), 36.6 (t), 36.5 (t), 27.3 (t) ppm; LRMS (ES^+) *m/z* (%) 209 $[M + H]^+$ (100), 231 $[M + Na]^+$ (5).[7]

4.6. Benzyloxycarbonylaminopropyl-3-isocyanate 3

Salt **3d** (1.75 g, 5.43 mmol) was dissolved in dichloromethane (100 mL) and saturated sodium bicarbonate solution (100 mL). The mixture was stirred at 0 ˚C in an ice bath for 30 min. The stirring was then stopped and the two phases were allowed to separate and phosgene solution (5.00 mL, 1.06 g, 10.80 mmol) was added to the organic layer and the stirring was resumed in an ice bath for 10 min then at room temperature for 17 hr. The organic layer was separated and the aqueous layer was washed with dichloromethane (2 x 150 mL). The combined organic layers were concentrated on *vacuo* to give isocyanate **3** (1.01 g, 4.31 mmol, 79 %) as a white solid, which was used without further purification. R_f (5 % methanol/ dichloromethane) 0.80; *v* (neat)/ cm^{-1} 3400 (m), 1700 (s), 1513 (m), 1370 (m), 1250 (s), 1153 (s); δ_H (300 MHz, CD_3CN) 7.36 (5H, br s, 5H Ar), 5.62 (1H, br s, *NH*Cbz), 5.05 (2H, br s, *CH*$_2$NHCbz), 3.12 (4H, m, $2CH_2$), 1.55 (2H, quint, *J* = 6 Hz, $CH_2CH_2CH_2$) ppm; δ_C (75 MHz, $CDCl_3$) 159.9 (s), 157.3 (s), 138.4 (s), 129.3 (d), 128.6 (d), 127.5 (d), 66.6 (t), 37.8 (t), 36.9 (t), 30.6 (t) ppm.[8]

4.7. Synthesis and characterization of {2-[3-(3-benzyloxycarbonylaminopropyl)ureido]pyridin-3-yloxy} *t*-butyl acetate 4

A solution of isocyanate **3** (0.12 g, 0.51 mmol) in dichloromethane (2 ml) was added to a stirred solution of amine **2** (0.1 g, 0.45 mmol) in dichloromethane (2 ml) and the mixture refluxed at 50˚C for 24 h. The solvent was evaporated under reduced pressure and the crude yellow oil was purified by column chromatography over silica gel using 0.5 % methanol: dichloromethane as eluent to give the urea **4** (0.08 g, 0.17 mmol, 39 %) as a yellow oil. R_f (5 % methanol/ dichloromethane) 0.60; *v* (neat)/ cm^{-1} 3254 (w), 2978 (w), 1673 (s), 1541 (s), 1410 (w), 1227 (s), 1151 (s); δ_H (300 MHz, $CDCl_3$); 9.49 (1H, br s, NH), 7.79 (1H, d, *J* = 5 Hz, H py), 7.52 (1H, br s, NH), 7.34 (5H, m, 5H Ar), 6.96 (1H, d, *J* = 8 Hz, H py), 6.81 (1H, dd, *J* = 8, 5 Hz, H py), 5.63

(1H, br s, *NH*Cbz), 5.09 (2H, s, CH_2Ph), 4.54 (2H, s, CH_2CO_2 *t*-Bu), 3.45 (2H, dt, *J* = 6, 6 Hz, CH_2NHCONH), 3.28 (2H, dt, *J* = 6, 6 Hz, CH_2NHCbz), 1.77 (2H, quint, *J* = 6 Hz, $CH_2CH_2CH_2$), 1.48 (9H, s, $3CH_3$) ppm; δ_C (75 MHz, $CDCl_3$); 166.8 (s), 156.7 (s), 155.5 (s), 144.5 (s), 140.9 (s), 138.2 (d), 136.9 (s), 128.4 (d), 128.0 (d), 127.9 (d), 118.3 (d), 116.3 (d), 83.2 (s), 66.5 (t), 66.4 (t), 38.1 (t), 36.8 (t), 30.5 (t), 28.1 (q) ppm; LRMS (ES^+) *m/z* (%) 459 $[M + H]^+$ (100), 481 $[M + Na]^+$ (52), 917 $[2 M + H]^+$ (12), 939 $[2 M + Na]^+$ (30).

4.8. Synthesis and characterization of {2-[3-(3-benzyloxycarbonylaminopropyl)ureido]pyridin-3-yloxy} acetic acid 5

A solution of urea **4** (0.5 g, 1.09 mmol) and trifluoroacetic acid, TFA, (20 % in dichloromethane, 30 ml) was stirred at room temperature for 2 h. Toluene (10 ml) was added and the solvent was evaporated under reduced pressure to give acid **5** (0.43 g, 1.07 mmol, 98 %) as a white solid which was used without further purification. R_f (15 % methanol/ dichloromethane) 0.40; mp = 150-153 ˚C; ν (film)/ cm^{-1} 3307 (m), 1686 (s), 1544 (s), 1211 (br); δ_H (300 MHz, CD_3CN); Two NH signals were broad, 8.61 (1H, br s, NH), 7.80 (1H, d, *J* = 6 Hz, H py), 7.62 (1H, d, *J* = 8 Hz, H py), 7.61 (5H, br s, 5H Ar), 7.18 (1H, dd, *J* = 8, 6 Hz, H py), 5.82 (1H, br s, *NH*Cbz), 5.07 (2H, s, CH_2Ph), 4.84 (2H, br s, CH_2COOH), 3.34 (2H, dt, *J* = 6, 6 Hz, CH_2NHCONH), 3.21 (2H, dt, *J* = 6, 6 Hz, CH_2NHCbz), 1.72 (2H, quint, *J* = 6 Hz, $CH_2CH_2CH_2$) ppm; δ_C (75 MHz, $CDCl_3$); 178.5 (s), 166.8 (s), 156.9 (s), 143.3 (s), 141.8 (s), 138.2 (d), 128.0 (s), 128.0 (d), 128.0 (d), 127.0 (d), 117.3 (d), 116.9 (d), 67.0 (t), 66.5 (t), 37.9 (t), 36.7 (t), 28.5 (t); LRMS (ES^+) *m/z* (%) 403 $[M + H]^+$ (100), 805 $[2 M + H]^+$ (8), 827 $[2 M + Na]^+$ (20).[7]

4.9. Synthesis and characterization of {2-[3-(3-aminopropyl)ureido]pyridin-3-yloxy} *t*-butyl acetate 6

A solution of urea **4** (0.17 g, 0.37 mmol) in methanol (5 ml) was hydrogenated over palladium on charcoal (0.02 g, 0.19 mmol) and the mixture was stirred over-night at room temperature. The catalyst was filtered over a celite pad, washed with methanol and the filtrate was concentrated in *vacuo* to give amine **6** (0.11 g, 0.34 mmol, 91 %) as a colorless oil which was used without further purification. ν (film)/ cm^{-1} 3431 (w), 2976 (w), 1747 (m), 1678 (s), 1545 (s), 1484 (s), 1227 (s), 1154 (s); δ_H (300 MHz, $CDCl_3$); 9.49 (1H, t, J = 6 Hz, NH), 7.77 (1H, d, J = 5, Hz, H py), 6.93 (1H, d, J = 8, Hz, H py), 6.79 (1H, dd, J = 8, 5 Hz, H py), 4.59 (2H, s, CH_2CO_2 *t*-Bu), 3.44 (2H, dt, J = 7, 7 Hz, CH_2NHCONH), 2.80 (2H, t, J = 7 Hz, CH_2NH_2), 1.79 (2H, quint, J = 7 Hz, $CH_2CH_2CH_2$), 1.49 (2H, br s, NH_2), 1.48 (9H, s, $3CH_3$) ppm; δ_C (75 MHz, $CDCl_3$); 166.7 (s), 155.2 (s), 144.5 (s), 140.8 (s), 138.1 (d), 118.1 (d), 116.4 (d), 83.1 (s), 66.3 (t), 37.3 (t), 36.4 (t), 28.8 (t), 27.9 (q) ppm; LRMS (ES^+) *m/z* (%) 325 $[M + H]^+$ (100), 649 $[2 M + H]^+$ (5), 974 $[3 M + Na]^+$ (20).[9]

4.10. Synthesis and characterization of (2-{3-[3-(2-{2-[3-(3-benzyloxycarbonylaminopropyl)-3-ureido]pyridin-3-yloxy} acetylamino)propyl]ureido}pyridin-3-yloxy) t-butyl acetate 7

Amine **6** (0.70 g, 2.16 mmol) was added to a solution of acid **5** (0.9 g, 2.23 mmol) in dried dichloromethane (10 ml), EDC (0.4 g, 2.09 mmol) and diisopropyl ethylamine (DIPEA) (0.4 ml, 0.29 g, 2.29 mmol) and the mixture was stirred at room temperature for 48 h under nitrogen. The solution was concentrated in *vacuo* and the crude oil was purified by column chromatography over silica gel using 2 % methanol: dichloromethane as eluent to give urea **7** (0.3 g, 0.42 mmol, 20 %) as a colorless oil. R_f (5 % methanol/ dichloromethane) 0.50; ν (film)/ cm^{-1} 3306 (m), 3063 (w), 2951 (w), 1689 (s), 1529 (s), 1453 (s), 1241 (s); δ_H (300 MHz, CD_3CN); 9.90 (3H, m, 3NH), 9.81 (1H, t, J = 4 Hz, NH), 9.51 (1H, t, J = 6 Hz, NH), 7.78 (2H, m, 2H py), 7.30 (5H, br s, 5H

Ar), 7.04 (1H, d, *J* = 7 Hz, H py), 6.82 (1H, dd, *J* = 7, 5 Hz, H py), 6.74 (2H, br s, 2H py), 5.75 (1H, br s, *NH*Cbz), 4.95 (2H, s, CH_2Ph), 4.45 (2H, s, CH_2CO_2 *t*-Bu), 4.39 (2H, s, CH_2CONH), 3.48 (2H, dt, *J* = 6, 6 Hz, $CH_2NHCOCH_2$), 3.40 (4H, dt, *J* = 6, 6 Hz, $2CH_2$NHCONH), 3.20 (2H, dt, *J* = 6, 6 Hz, CH_2NHCbz), 1.70 (4H, br s, $2CH_2CH_2CH_2$), 1.37 (9H, s, $3CH_3$) ppm; δ_C (75 MHz, $CDCl_3$); 167.6 (s), 166.5 (s), 160.1 (s), 158.1 (s), 157.1 (s), 145.3 (s), 144.6 (s), 142.0 (s), 141.3 (s), 137.9 (d), 137.6 (d), 136.9 (s), 128.6 (d), 128.4 (d), 128.1 (d), 119.4 (d), 117.8 (d), 116.6 (d), 116.4 (d), 82.8 (s), 67.8 (t), 66.9 (t), 66.7 (t), 38.2 (t), 36.5 (t), 36.0 (t), 34.4 (t), 30.9 (t), 29.6 (t), 28.1 (q) ppm; LRMS (ES^+) *m/z* (%) 709 $[M + H]^+$ (100), 731 $[M + Na]^+$ (45).

4.11. Synthesis and characterization of (2-{3-[3-(2-{2-[3-(3-Benzyloxycerbonylaminopropyl) ureido]pyridin-3-yloxy}acetylamino)propyl]ureido}pyridin-3-yloxy) acetic acid 8

A solution of urea **7** (0.1 g, 0.14 mmol) in trifluoroacetic acid, TFA, (20 % in dichloromethane, 15 ml) was stirred at room temperature for 2 h. Toluene (10 mL) was added and the solvent evaporated under reduced pressure to give acid **8** (0.08 g, 0.12 mmol, 87 %) as a white solid which was used without further purification. R_f (12 % methanol/ dichloromethane) 0.50; ν (film)/ cm^{-1} 3307 (br), 3067 (br), 1671 (s), 1573 (s), 1182 (s), 1134 (s); ^{1}H NMR and MS confirmed the formation of acid **8**; δ_H (300 MHz, CD_3CN); 10.30 (2H, br s, 2NH), 8.64 (1H, br s, NH), 8.41 (1H, br s, NH), 8.10 (1H, br s, NH), 7.85 (2H, m, H py), 6.62 (2H, m, 2 H py), 7.58 (5H, br s, 5 H Ar), 7.18 (2H, m, 2 H py), 5.85 (1H, br s, NH), 5.04 (2H, br s, CH_2Ph), 4.83 (2H, br s, CH_2COOH), 4.66 (2H, s, CH_2CONH), 3.33 (6H, br s, $3CH_2$), 3.18 (2H, dt, *J* = 6, 6 Hz, CH_2NHCbz), 1.71 (4H, quint, *J* = 6 Hz, $2CH_2CH_2CH_2$) ppm; LRMS (ES^+) *m/z* (%) 653 $[M + H]^+$ (100).

4.12. Synthesis and characterization of (2-{3-[3-(2-{2-[3-(3-Aminopropyl)ureido]pyridin-3-yloxy} acetylamino)propyl]ureido}pyridin-3-yloxy) acetic acid 9

A solution of urea **7** (0.1 g, 0.15 mmol) in methanol (5 ml) was hydrogenated over palladium on charcoal (0.008 g, 0.075 mmol) and the mixture stirred at room temperature over-night. The catalyst was filtered through a celite pad and washed with methanol and the filtrate was concentrated in *vacuo* to give amine **9** (0.075 g, 0.15 mmol, 95 %) as a colorless oil, which was used without further purification. The mass spectroscopic data confirmed the formation of the product, LRMS (ES^+) *m/z* (%) 519 $[M + H]^+$ (100), 541 $[M + Na]^+$ (20).

4.13. Synthesis and characterization of 9,10,11,12,23,24,25,26-octahydro-6H,20H-dipyrido[3,2-b:3,2-n][1,13,4,6,10,16,18,22]dioxahexaazacyclotetracosine-7,13,21,27(8H,14H,22H,28H)-tetrone 1

Urea **9** (0.075 g, 0.15 mmol) was dissolved in a solution of DMF: DCM (1:1 ratio, 5 mL) and EDC (0.03 g, 0.16 mmol) was added and the mixture stirred at room temperature for 30 min. Diisopropyl ethylamine (27 µl, 0.14 mmol) was then added and the mixture stirred at room temperature for 36 h under nitrogen. The solvent was evaporated under reduced pressure and the crude solid was purified by column chromatography over silica gel using 2 % methanol: dichloromethane as eluent to give the macrocycle **1** (0.016 g, 0.032 mmol, 30 %) as a white solid. Crystals of macrocycle **1** were grown in 1:1 chloroform/methanol: diethyl ether and the full X-ray crystal structure data are given as supplementary material. R_f (5 % methanol/ dichloromethane) 0.50; mp = 300-301 ˚C; IR *v* (film) cm^{-1} 3212 (s), 3084 (w), 1650 (s), 1547 (s), 1489 (s), 1414 (s); δ_H (300 MHz, CD_3CN); 10.20 (1H, br s, NH), 9.88 (1H, br s, *NH*$COCH_2O$), 9.58 (1H, br s, *NH*CONH), 7.76 (2H, d, *J* = 5 Hz, 2H py), 7.03 (2H, d, *J* = 8 Hz, 2H py), 6.83 (2H, dd, *J* = 8, 5 Hz, 2 H py), 4.40 (4H, br s, 2*CH*$_2$CONH), 3.54 (4H, m, 2*CH*$_2$NHCOCH_2), 3.40 (4H, m, 2*CH*$_2$NHCONH), 1.73 (4H, quint, *J* = 6 Hz, 2CH_2*CH*$_2$$CH_2$) ppm; δ_C (75 MHz, $CDCl_3$); 166.9 (s), 158.2 (s), 143.6 (s), 141.2 (s), 137.6 (d), 118.2 (d), 117.1 (d), 67.5 (t), 35.9 (t), 34.3 (t),

29.2 (t) ppm; LRMS (ES^+) *m/z* (%) 501 $[M + H]^+$ (80), 523 $[M + Na]^+$ (100), 1023 $[2 M + Na]^+$ (8).[9]

5. Conclusions

An *N*-Pyridyl-urea macrocycle **1** has been found to be easy to synthesize and was obtained by a final cyclisation in 30 % yield. It showed the expected conformation **X** with the intramolecular hydrogen bonding between the urea *N*-H and pyridyl-*N* as confirmed by both its X-ray crystal structure and its ^1H NMR spectrum. The tetrafluoroborate, chloride, bromide and sulphate salts of macrocycle **1** were prepared following the same method as used for their acyclic urea analogue, but these salts were sparingly soluble in a range of solvents and were only soluble in DMSO. Attempts to solubilize in DMSO led to deprotonation of the salts giving back the neutral macrocycle.

Acknowledgements

This research project was supported by a grant from the "Research Center of the Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

Conflict of interest

Authors declare no conflict of interest.

References

1. R. Arienzo and J.D. Kilburn, *Tetrahedron*, 2002, **58**, 711.

2. U. Sakee and C. Nasuk, *Carbohydr. Res.*, 2010, **345**, 1222.

3. J.A. Bristol, G. Irwin and R.G. Lovey, *Synthesis*, 1981, **12**, 971.

4. H. Deborah, S. Michael, G. Sarah and K. Tomohiko, *PCT Int. Appl*., 2014, WO 2014159938 A1 20141002.

5. K.B. Jensen, T.M. Braxmeier, M. Demarcus, J.G. Frey and J.D. Kilburn, *Chem. Euro. J*., 2002, **8**, 1300.

6. M. Frankel, D. Ladkany, C. Gilon and Y. Wolman, *Tetrahedron Lett*., 1966, **7**, 4765.

7. M. Bodansky and A. Bodansky, *The practice of peptide synthesis*. Berlin, New York, Springer-Verlag, 1984.

8. S.N. James, D.L. Holmes , G. Noronha , E.M. Smith , T.M. Nguyen and S. Huang, *J. Org. Chem*., 1996, **61**, 3929.

9. B.R. Linton, A.J. Carr, B.P. Orner and A.D. Hamilton, 2000, *J. Org. Chem.*, 2000, **65**, 1566.