HETEROCYCLES, Vol. 73, 2007, pp. 617 - 625. © The Japan Institute of Heterocyclic Chemistry
Received, 29th June, 2007, Accepted, 24th August, 2007, Published online, 24th August, 2007. COM-07-S(U)40

SYNTHESIS OF NOVEL UREA BRIDGED MACROCYCLIC MOLECULES USING BTC

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Abstract – Cyclic ureas have interesting pharmacological properties that have led to their use as analogs of bioactive compounds. Several synthetic routes for urea formation by cyclization of diamines are known, based on the nucleophilic reaction of the amines with phosgene or phosgene derivatives. We have developed a procedure for a triphosgene mediated, on-resin urea cyclization. Four novel urea macrocyclic molecules were synthesized in order to demonstrate the feasibility of this method.

INTRODUCTION

Urea is a common structural element in many natural products and synthetic bioactive compounds. Over the past decade, many urea cyclic molecules have been synthesized, among them Human Immunodeficiency Virus (HIV) protease inhibitors[1-3] and enkephalin analogs. Procedures for the synthesis of cyclic ureas are described in the literature,[4] most of them are based on the nucleophilic reaction of diamines with phosgene or related compounds.[5-8]

Few procedures have been introduced for urea cyclization on solid support. [4, 9-11] The synthesis of cyclic ureas from diamines, using phosgene derivatives can lead to the formation of bis-isocyanates, which is predominant when a strong activating agent like Bis(trichloromethyl)carbonate (BTC) is used.

The low toxicity of BTC, compared to other phosgene derivatives, and its stable solid form at room temperature, makes it an attractive reagent for many synthetic applications.[12, 13] BTC has been used for the synthesis of cyclic ureas in solution [1, 3, 14-16] and for BTC mediated solid phase urea cyclization.[10]

We introduce a new procedure for the solid phase preparation of urea bridged macrocyclic compounds, which can be generalized to other urea cyclic compounds. This procedure utilizes N^{α}-glycine building units previously developed in our laboratory, on resin reductive alkylation of Alloc protected amino alkyl

aldehydes for the preparation of the precyclic diamines and on resin cyclic urea formation using BTC.

RESULTS AND DISCUSSION

Mechanistic studies of HIV-1 infection have shown that the interaction of T-cell surface protein CD4 with the viral protein gp120 is crucial for the penetration of the virus into the cytoplasm.[17, 18] Two important amino acid residues, arginine (Arg59) and phenylalanine (Phe43) of the CD4 extra cellular domain take part in the CD4-gp120 interaction. [19-22] In the current study we designed a small library of macrocyclic molecules with varying ring sizes that preserve the active pharmacophores (guanidine and phenyl) in attempt to inhibit the interaction between gp120 and CD4 (Figure 1). The library was synthesized in accordance with the recently reported approach for the design of macrocyclic molecules libraries with conformational diversity. [23]

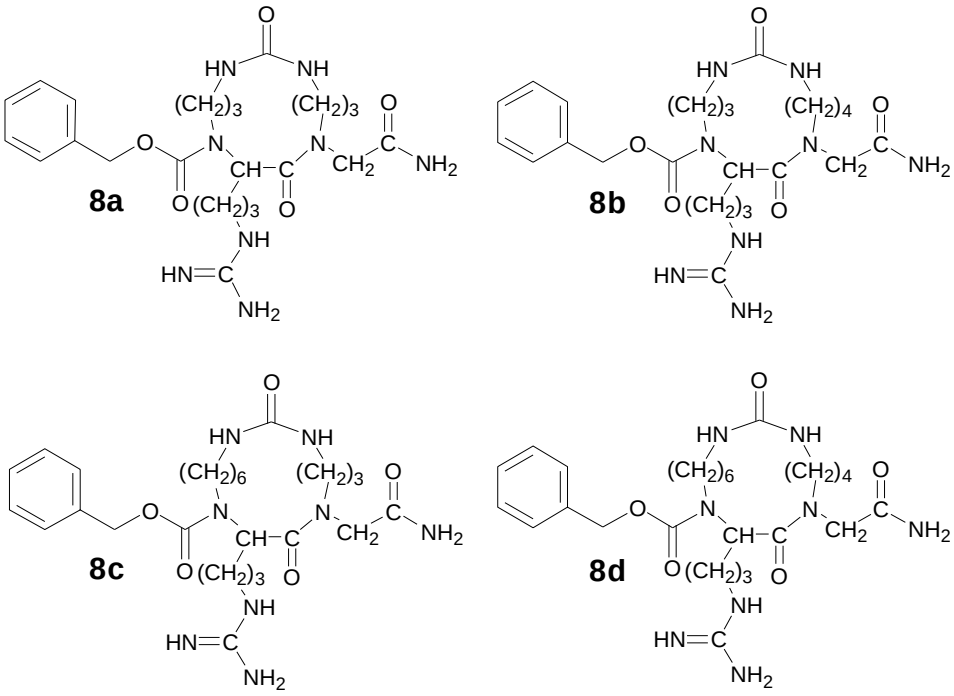

Figure 1 Structures of the urea macrocyclic library

Based on a method introduced by Gazal *et al*.,[24] we have developed a high yield procedure for the synthesis of Alloc protected amino aldehyde to be used for on resin reductive alkylation. This procedure utilizes the *N,O*-dimethyl hydroxyl amine hydrochloride reagent [25] for the preparation of the Alloc amino aldehydes.

3-Aminopropionic acid and 6-Aminohexanoic acid were treated with *N*-(allyloxycarbonyloxy)-succinimide (Alloc-OSu) to give 3-allyloxycarbonylaminopropionic acid **1a** and 6-allyloxycarbonyl-aminohexanoic acid **1b** (Figure 2). The protected amino n-alkyl acids **1a** and **1b** were activated with

thionyl chloride to obtain the corresponding acyl chlorides which were reacted immediately with *N*,*O*-dimethylhydroxylamine hydrochloride to form [2-(methoxymethylcarbamoyl)ethyl]carbamic acid allyl ester **2a,** and [5-(methoxymethylcarbamoyl)pentyl]carbamic acid allyl ester **2b**. Reduction of **2a** and **2b** with $LiAlH_4$ produced 3-allyloxycarbonylaminopropanal **3a** and 6-allyloxycarbonylaminohexanal **3b** respectively. The aldehydes were used within 48 h of their preparation.

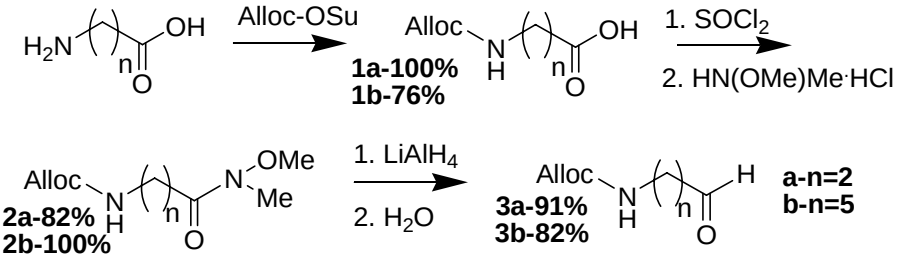

Figure 2 Synthesis of 3-Allyloxycarbonylaminopropanal and 6-Allyloxycarbonylaminohexanal.

The synthesis of compounds **8a-d** was performed according to Figure 3.

Fmoc -[γ-*N*(Alloc)-propyl]Gly-OH and Fmoc-[δ-*N*(Alloc)-butyl]Gly-OH were synthesized according to an improved procedure (unpublished results) and were preloaded on a Rink Amide MBHA resin using a modification of a previously described procedure[26] to give **4a** and **4b** respectively. Introduction of *N*-α-Fmoc-N^{γ}-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-L-arginine (Fmoc-Arg(Pmc)-OH) to the secondary amine was carried out under special conditions to give **5a** and **5b**.

A simple method for direct on-resin reductive alkylation was developed, avoiding di-alkylation of the amine. Following removal of the Fmoc protecting group from **5,** the appropriate aldehydes **3a** and **3b** dissolved in dry trimethylorthoformate were added to the resin-bound unprotected amine to form the corresponding Schiff base which was reduced with $NaBH(OAc)_3$ to generate **6**. Mass spectroscopy analysis performed after the reduction step confirmed that no di-alkylation occurred (results not shown), indicating that the procedure has an advantage over other methods used for alkylation of amines. This method offers a general approach for the introduction of functional moiety to any resin bound primary amine. The secondary amine **6** was treated with benzyloxycarbonyl chloride (Z-Cl) with the purpose of mimicking the aromatic character of the phenylalanine residue. The Alloc protecting group was readily removed in the usual manner using tetrakis(triphenylphosphine)palladium(0)[27] to form the precyclic diamine **7**. The major weakness of on-resin cyclization by simultaneous introduction of BTC and tertiary base to diamine **7** is the formation of bis-isocyanates **9** as a by-product (Figure 4). Our early attempts to optimize simultaneous BTC/tertiary base mediated urea cyclization based on previously reported procedures led to the formation of a mixture of products, including traces of the desired cyclic compound and the non-cyclic diamine by-product.

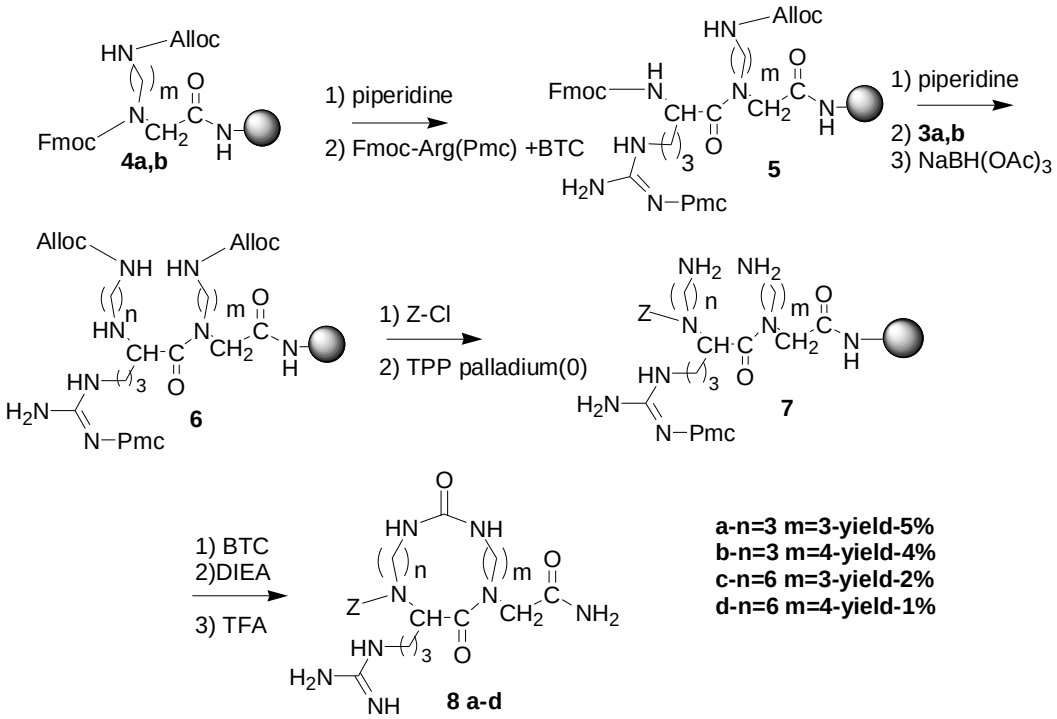

Figure 3 Synthesis of urea heterocyclic molecules **8a-d**

A two steps method was developed in order to prevent the formation of the bis-isocyanates **9**. First, 1/3 equivalents of BTC were added to the diamine and the reaction vessel was agitated for 2 hours. Then, 2 equivalents of ethyldiisopropylamine were added and the mixture was agitated for 16 h. We assume that in the first step, one of the amines reacted with BTC to create the mono isocyanate and that the HCl released in this reaction was trapped by the second proximate amine (**10** in Figure 4). The ammonium ion formed is less active, hence, decreases the reaction rate with other phosgene molecules and reduces the possibility of forming the bis-isocyanate **9**. Addition of the tertiary base after 2 h, to free the nucleophilc amine, shifts the equilibrium toward the formation of **11.** Extensive shaking for additional 16 h at room temperature was needed to complete the cyclization. An accurate amount of BTC is essential to ensure the formation of only one isocyanate group for each molecule. An excess of BTC causes the formation of bis-isocyanate, while less then 0.33 equivalents of BTC results in the formation of the non-cyclic diamine as a major product. After cleavage from the resin, compounds **8a-d** were isolated as the acetate salts, purified and characterized by mass spectroscopy (MS). Characterization by MS/MS experiments confirmed the presence of the urea segment. We report a procedure for synthesizing urea macrocyclic molecules by using a novel BTC mediated on-resin cyclization. This method is straightforward and can be used to synthesize both symmetrical and nonsymmetrical cyclic ureas in various ring sizes on solid support. We further present an improved procedure for on-resin reductive alkylation that can be applied for on-resin alkylation of amino acids or any primary amines attached to solid support. These new

methods are powerful tools for the synthesis of small molecule libraries and allow the screening of the conformational space for discovery of bioactive compounds.

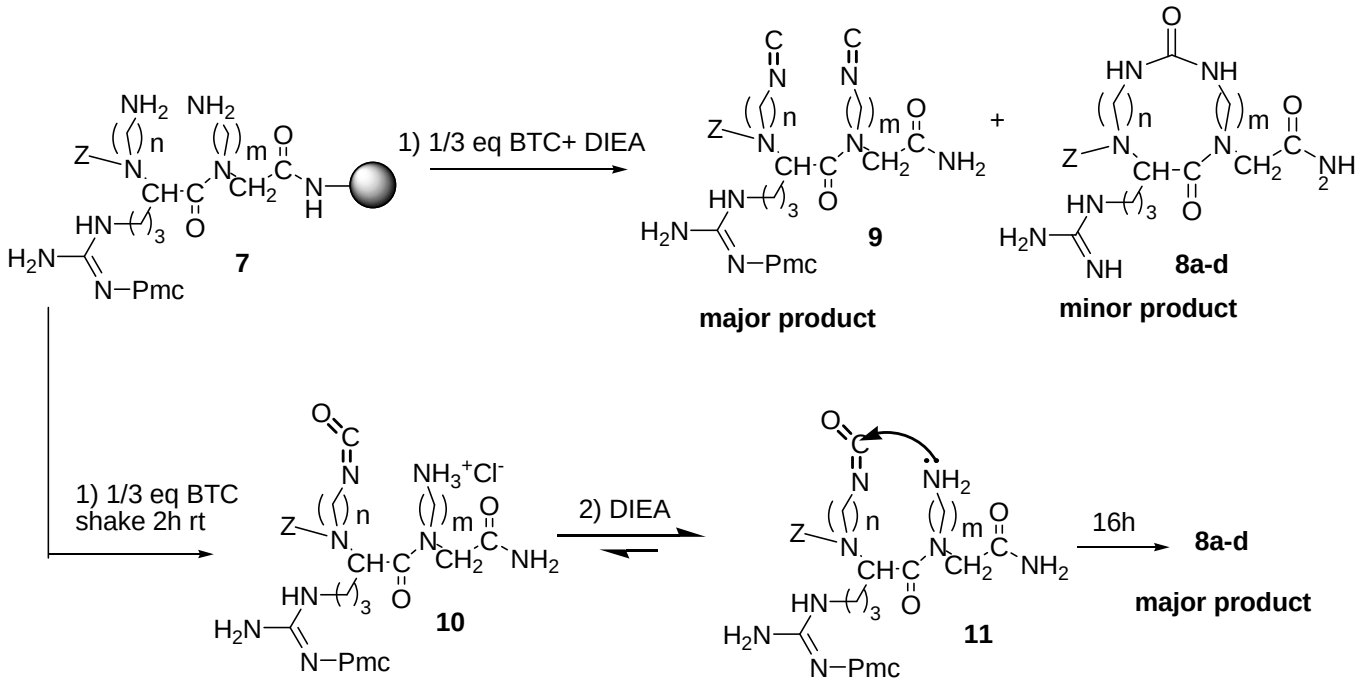

Figure 4: The two steps urea cyclization procedure vs. the one step cyclization procedure

EXPERIMENTAL

Abbreviations used in the text: Acetonitrile, ACN; Allyloxycarbonyl, Alloc; Bis(trichloromethyl)carbonate, BTC; Dichloromethane, DCM; ethyldiisopropylamine, DIEA; Dimethylformamide, DMF; Ethyl acetate, EA; 9-Fluoromethylmethoxycarbonyl, Fmoc; Mass Spectroscopy, MS; 1-Methyl-2-pyrrolidone, NMP; 4-Methylmorphidine, NMM; *N*-hydroxysuccinimide, OSu; Petroleum ether, PE; Trifluoroacetic acid, TFA; Trimethylorthoformate, TMOF.

Reactions in solution phase were monitored by thin-layer chromatography (TLC) on Merck F245 60 silica gel plates. The NMR spectra were obtained on a BRUKER AMX-300 MHz at 295°K. Solid phase reactions were monitored using MS. MS and MS/MS experiments were done on a Qtof II micromass instrument. Preparative RP-HPLC purifications were carried out on a Merck-Hitachi–L6200A intelligent pump with a Jasco UVDEC-100-V (UV) detector. Solvents A (0.1 % TFA in water) and B (0.1 % TFA in ACN) were used in linear gradient (95% A→95% B in 50 min). The flow rate was 9 mL/min, using XTerra® RP-C18 10 μm 19 x 300 mm column. Analytical RP-HPLC was carried out on an analytic HPLC-waters 2695 separations modules with a waters PDA 2996 detector. Solvents A (0.1 % TFA in water) and B (0.1 % TFA in ACN) were used in linear gradient (95% A→95% B in 35min). The flow

was 1 mL/min using a - XTerra® Anal RP-C8 3.5 μm 4.6 x 150 mm column.

Synthesis of Alloc protected amino aldehydes

Allocilation of N^{ω}-amino acid: Into a round bottom flask with a magnetic stirrer and cooled in an ice bath was added NH_2-(CH_2)n-COOH (1 mol) dissolved in 4N NaOH solution (250 mL). A solution of *N*-(allyloxycarbonyloxy)succinimide (Alloc-OSu) (1.3 mol) in 4N NaOH solution (250 mL) was added in eight portions (after each addition the Alloc-OSu solution was vigorously shaking for a few minutes to dissolve Alloc-OSu before the next addition). The pH was adjusted to 10 with 4N NaOH solution (about 100 mL). Reaction progress was monitored by TLC (PE:EA 1:1). The reaction mixture was stirred overnight before being diluted with water (450 mL). The aqueous layer was washed with PE (3 × 300 mL) and acidified to pH=1 with conc. HCl and product was extracted with EA (3 × 300 mL). The combined EA extract was dried with $MgSO_4$, filtered and evaporated to yield clear oil. No further purification was needed for the next step.

3-Allyloxycarbonylaminopropionic acid (1a):

Yield: 100%; TLC pure; ^{1}H NMR ($CDCl_3$), δ/ppm 2.61 (t, *J*=5.4 Hz, 2H), 3.47 (q, *J*=6.0 Hz, 2H), 4.57 (d, *J*=5.1 Hz, 2H), 5.26 (m, 2H) 5.93 (m, 1H).

6-Allyloxycarbonylaminohexanoic acid (1b):

Yield: 76**%;** TLC pure; ^{1}H NMR ($CDCl_3$), δ/ppm 1.38 (m, 2H), 1.53 (m 2H), 1.66(m, 2H), 2.36 (t, *J*=5.3 Hz,2H), 3.19 (q, *J*=6.0 Hz, 2H), 4.58 (d, *J*=5.1 Hz, 2H), 5.26 (m, 2H), 5.92 (m, 1H).

Synthesis of Alloc-amino n-alkyl Hydroxamate (2a,d): In a round bottom flask a mixture of Alloc-NH-(CH_2)n-COOH **1a,b** (0.205 mol) and SO_2Cl_2 (2 mol), were dissolved in dry CH_2Cl_2 (1000 mL) and were refluxed for 2 h under argon. Another portion of SO_2Cl_2 (1 mol) was added, and the solution was refluxed for an additional 1 h. After cooling to rt, the solvent and excess of SO_2Cl_2 were removed by evaporation. Dry CH_2Cl_2 (450 mL) was added to the oily residue and evaporated again. This was repeated three more times, to remove excess of SO_2Cl_2. The yellow oil was dissolved in dry CH_2Cl_2 (500 mL) and set to stir in an ice bath, before adding *N,O*-dimethyl hydroxyl amine hydrochloride (0.227 mol) and Et_3N (40-60 mL). The solution was stirred for 1 h keeping the solution alkaline by adding Et_3N if necessary. Most of the solvent was evaporated, and the residue was dissolved in brine (200 mL) and extracted to a mixture of Et_2O: CH_2Cl_2 (1:1 200 mL). The organic layer was then dried with Na_2SO_4, filtered, and evaporated to obtain yellow oil. The hydroxamates were used for the next step without further purification.

[2-(Methoxymethylcarbamoyl)ethyl]carbamic acid allyl ester (2a):

Yield: 82%; TLC pure; ^{1}H NMR ($CDCl_3$), δ/ppm 2.66 (t, *J*=5.4 Hz, 2H), 3.18 (s, 3H) 3.48 (q, *J*=5.7 Hz, 2H),3.68 (s, 3H) 4.55 (d, *J*=5.1 Hz, 2H), 5.25 (m, 2H) 5.91 (m, 1H).

[5-(Methoxymethylcarbamoyl)pentyl]carbamic acid allyl ester (2b):

Yield: 100%; TLC pure; ^{1}H NMR ($CDCl_3$), δ/ppm 1.38 (m, 2H), 1.53 (m, 2H), 1.65(m, 2H), 2.43 (t, *J*=7.5 Hz, 2H), 3.18 (m, 5H), 3.68 (s, 2H), 4.56 (d, *J*=5.1 Hz, 2H), 5.28 (m, 2H), 5.92 (m, 1H).

Reduction of Alloc N^{ω}-amino alkyl Hydroxamates to aldehydes:

A stirred solution of the Weinreb amides **2a** and **2b (**0.014 mol) dissolved in dry Et_2O (140 mL) was cooled in an ice bath under Argon. $LiAlH_4$ (0.028mol) was added in portions and the reaction was stirred until completion (~40 min monitored by TLC (PE:EA 1:1)) EA (250 mL) was added slowly, followed by addition of 1M $KHSO_4$ (250 mL). The solution was left to stir for 30 min at rt. The organic layer was separated and washed with 1M $KHSO_4$ (2 × 150 mL), and saturated aq NaCl, (1 x 150 mL), dried over Na_2SO_4, filtered and evaporated. The resulting colorless oil was dried overnight under vacuum and was kept under Argon at -20°C.

3-Allyloxycarbonylaminopropanal (3a): Yield: 91%; TLC –pure; ^{1}H NMR ($CDCl_3$), δ/ppm 2.74 (t, *J*=5.8 Hz, 2H), 3.47 (q, *J*=3.3 Hz, 2H), 4.55 (d, *J*=5.3 Hz, 2H), 5.26 (m, 2H) 5.89 (m, 1H), 9.81 (s, 1H).

6-Allyloxycarbonylaminohexanal (3b): Yield: 86%; TLC –pure; ^{1}H NMR ($CDCl_3$), δ/ppm 1.34 (m, 2H), 1.53 (m, 2H), 1.65(m, 2H), 2.42 (m, 2H), 3.2 (q, *J*=6.7 Hz, 2H), 4.54 (d, *J*=5.3 Hz, 2H), 4.8 (s, 1H), 5.27 (m, 2H), 5.91 (m, 1H), 9.76 (s, 1H).

Synthesis of urea macromolecules

Solid phase procedures: Swelling: Swelling of the resin by shaking the resin for at least 2 h in 1-methyl-2-pyrrolidinone (NMP). Fmoc deprotection: shaking the resin in a solution of 20% Piperidine in NMP 2x30min, followed by resin wash in NMP (× 5) and CH_2Cl_2 (× 2). Fmoc-N^{α}-[ω-N(Alloc)n-alkyl]Gly-OH building units Coupling: 3eq protected amino acid and 1eq BTC dissolved in DCM were cooled in an ice bath. 7eq of DIEA were added to the resin and 7eq of 2,4,6-collidine were added to the cooled mixture. The resulting solution was preactivated for 1 min before added to the resin. After stirring for 1 h at rt, the resin was washed with CH_2Cl_2 (× 5) and NMP (× 2). Special BTC coupling conditions (for Fmoc-Arg(Pmc)-OH): 5 eq Fmoc-Arg(Pmc)-OH and 1.33 eq BTC were dissolved in dibromomethane, cooled in an ice bath and 20 eq of 2,4,6 collidine were added to the mixture. The mixture was preactivated for 1min, and was added to the preheated resin (50°C). After stirring for 3 h at 50°C the resin was washed with CH_2Cl_2 (x 5) and NMP (× 2). Alloc deprotection: A mixture of 92.5% CH_2Cl_2 /2.5% AcOH/2.5% NMM was added to the pre-dried resin under Argon and the reaction vessel was covered with aluminum foil. Tetrakis(triphenylphosphine)palladium(0) (1 gr /0.75 gr resin) was added to the vessel. After 2 h the reaction was stopped and the resin was washed with $CHCl_3$ (× 8). Reductive alkylation: A solution of 1% AcOH in TMOF (1 mL solution/ 0.2 gr resin) was added to pre-dried resin under Argon and treated with 1 equivalent of the appropriate aldehyde dissolved in TMOF (2 mL /0.2 gr resin). The mixture was left to stand under Argon atmosphere for 2 min and then stirred.

After 5 min 1 equivalent of sodium triacetoxy borohydride ($NaBH(OAc)_3$) was added to the mixture and reaction vessel was agitated for 3 h at rt. The resin was washed with: MeOH(× 3) 10% H_2O/ MeOH(× 2) 1 % AcOH/ H_2O(× 4) 10% H_2O/ MeOH(× 2) MeOH(× 3) CH_2Cl_2 (× 2) DMF(× 2). Benzyloxycarbonyl chloride (Z-Cl) (20 eq) dissolved in DMF was cooled in an ice bath. 20 eq of 2,4,6 collidine were added and the mixture introduced to the preheated resin. The reaction was stirred for 3 h at 55 ºC before it was washed with DMF (× 2) CH_2Cl_2 (× 2). Urea cyclization: resin was swelled in CH_2Cl_2 and 1/3 eq of BTC was added and agitated for 2h, then 2eq of ethyldiisopropylamine was added and left to stir for 16h before it was washed with CH_2Cl_2 (× 2). Cleavage: The cleavage was performed with a mixture of 95% TFA/2.5% Triisopropylsilane/2.5% H_2O at the ratio of 7 mL (cleavage mixture) for 150 mg (resin). The mixture was pre-cooled in an ice bath for 30 min and then added to the resin. Cooled for 30 min, and then shaken at rt for 3 h. The mixture was filtered and TFA was evaporated under stream of dry nitrogen, the remaining orange oil was dissolved in a mixture of ACN:H_2O 1:1 and lyophilized. Reactions were monitored by standard chloranil test and by small cleavage.

The macromolecules were isolated as the acetate salt and showed a mixture of conformers:

8a: Prepared from 200 mg of Fmoc-Rink-Amide MBHA resin. Yield 3.4mg (5%); HPLC purity > 95%, *R*t 11.8(36), 12.7(63); MS exact mass calculated for $C_{25}H_{40}N_8O_7$ 564.30 (M+AcOH) Found 564.47.

8b: Prepared from 200 mg of Fmoc-Rink-Amide MBHA resin. Yield 2.8mg (4%). HPLC purity > 99%, *R*t 13.6(50.7), 13.9(49.3); MS exact mass calculated for $C_{26}H_{42}N_8O_7$ 578.32 (M+AcOH) Found 578.53.

8c: Prepared from 200 mg of Fmoc-Rink-Amide MBHA resin. Yield 1.5mg (2%). HPLC purity > 99%, *R*t 15.7(72), 16.3(14), 16.6(13); MS exact mass calculated for $C_{28}H_{46}N_8O_7$ 606.35 (M+AcOH) Found 606.33.

8d: Prepared from 200 mg of Fmoc-Rink-Amide MBHA resin. Yield: 1mg (1%). HPLC purity > 95%, *R*t 16.1(49), 17.1(26), 17.5(25); MS exact mass calculated for $C_{29}H_{48}N_8O_7$ 620.36 (M+AcOH) Found 620.33.

ACKNOWLEDGEMENTS

This paper is dedicated to the memory of the late Prof. Ivar Ugi.

The authors would like to thank Dr. Ofra Moshel for her help with the MS and MS/MS analysis.

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