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A solution to the component instability problem in the preparation of peptides containing C2-substituted *cis*-cyclobutane β-aminoacids: synthesis of a stable rhodopeptin analogue

Olivier Roy, Sophie Faure and David J. Aitken*

Laboratoire SEESIB-CNRS, Département de Chimie, Université Blaise Pascal, Clermont-Ferrand II, 24 Avenue des Landais, 63177 Aubière cedex, France

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Abstract—Despite the inherent instability of C2-substituted *cis*-cyclobutane β-aminoacids, incorporation of such residues into peptides is shown to be possible through use of a 1-amino-2-(hydroxymethyl)cyclobutane derivative as a stable β-aminoacid surrogate. This synthetic strategy was validated by the synthesis of a rhodopeptin analogue.
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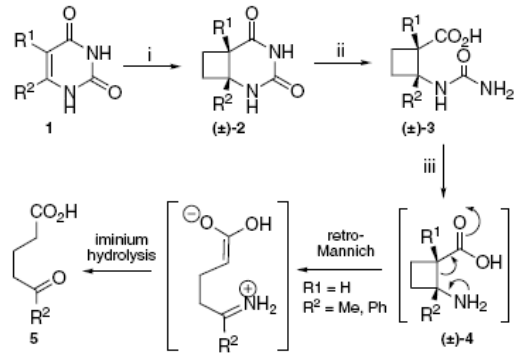
The incorporation of conformationally restricted β-aminoacids into linear or cyclic peptides can dramatically influence their secondary and tertiary structures, and therefore their biological activities.[1] Alicyclic β-aminoacids are particularly attractive building-blocks for imposing constraints upon peptides.[2] In contrast with intensive work on cyclohexane, cyclopentane and cyclopropane β-aminoacids,[2,3] structural studies on peptides containing cyclobutane β-aminoacids are in their infancy.[4] Ortuño's work on the incorporation of the parent compound *cis*-2-aminocyclobutane-1-carboxylic acid into small oligomers produced encouraging preliminary results and indicated a strong tendency to impose well-defined secondary structure.[4] This parent molecule can be prepared in racemic[5,4d] or enantiomerically pure[6,4d] form, but the synthesis of ring-substituted cyclobutane β-aminoacids remains rare.[7]

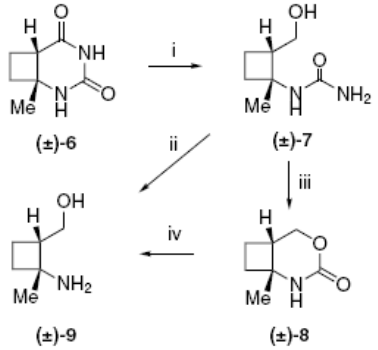
We recently described the synthesis of a series of C1- or C2-substituted *cis*-2-aminocyclobutane-1-carboxylic acids **4** based on the photochemical [2+2] cycloaddition of substituted uracils **1** with ethylene, to give **2**, followed by two-step heterocycle transformation via a ureidoacid intermediate **3** (Scheme 1).[8] This work revealed the inherent instability of *cis*-cyclobutane β-aminoacids substituted at C2 with electron-donating substituents, even weak ones such as alkyl or aryl. Rapid ring opening occurs by a retro-Mannich reaction leading to the corresponding acyclic δ-ketoacids **5**.[9] In this letter, we provide a solution to this component instability problem in the preparation of peptides containing *cis*-cyclobutane β-aminoacids, and illustrate its application to the synthesis of a constrained analogue of a rhodopeptin.



Scheme 1. Reagents and conditions: (i) hν, ethylene, acetone/water 1:1, rt; (ii) NaOH 0.5 M, rt; (iii) $NaNO_2$, HCl 3.5 M, rt.

Since C2-substituted *cis*-cyclobutane β-aminoacids cannot be used as such in peptide synthesis, it was considered appropriate to use a stable surrogate. A primary alcohol as a masked carboxylic acid function seems to be the most convenient. 1-Amino-2-(hydroxymethyl)-cyclobutane derivatives have been described with no

* Corresponding author. Tel.: +33 4 73407184; fax: +33 4 73407717; e-mail: David.Aitken@univ-bpclermont.fr



Scheme 2. Reagents and conditions: (i) $NaBH_4$, H_2O, rt, then H^+ resin, 72%; (ii) $NaNO_2$, HCl 3.5 M, rt, 15%; (iii) $NaNO_2$, HCl 3.5 M, THF, rt, 76%; (iv) NaOH, EtOH/H_2O, reflux, 67%.

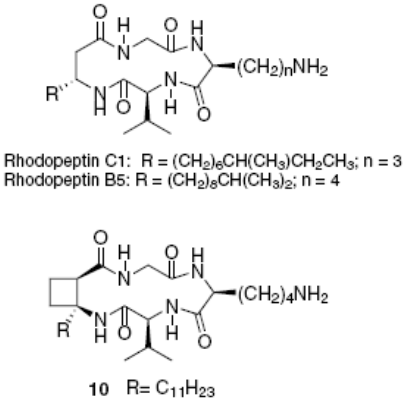


Figure 1. Structures of rhodopeptins and the cyclobutane analogue **10**.

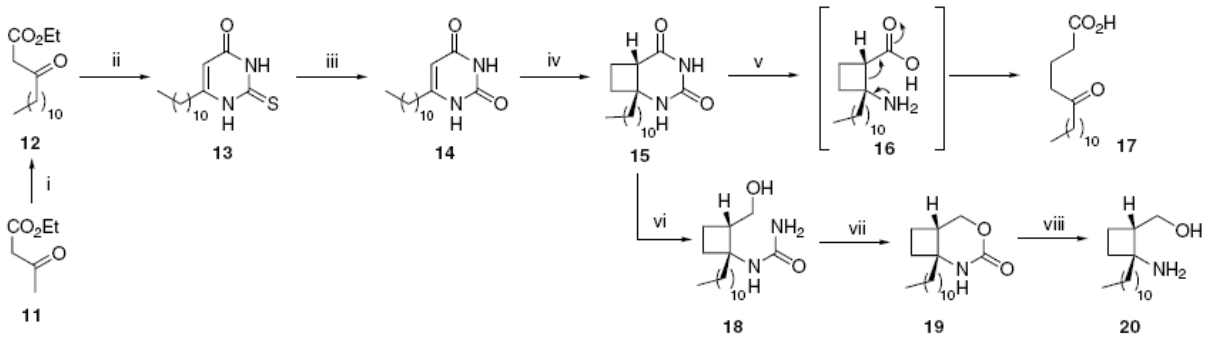
indication of instability.[10] First, we verified using a model compound that rapid access to the corresponding aminoalcohol from a cyclobutane dihydrouracil derivative was feasible. Compound **6** was obtained by a [2+2] photocyclization reaction between 6-methyluracil and ethylene, as previously described.[8] Reduction using an excess of sodium borohydride in water at room temperature,[11] followed by acidification with an acidic ion exchange resin, gave ureidoalcohol **7** in 72% yield (Scheme 2). Unexpectedly, the diazotization reaction under standard conditions (1 equiv $NaNO_2$ in 3.5 M HCl solution)[8] gave aminoalcohol **9** in poor yield (15%) together with cyclic carbamate **8**. The best compromise to obtain the cyclobutane aminoalcohol was to generate selectively the cyclic intermediate **8** via a diazotization reaction carried out in tetrahydrofuran as co-solvent (76% isolated yield). The cyclic carbamate was hydrolyzed in basic conditions to provide aminoalcohol **9** in good yield (67%).[12] In contrast to its β-aminoacid homologue, **9** is perfectly stable.

Rhodopeptins are cyclic lipopeptides recently isolated from the bacterial species *Rhodococcus* sp. Mer-N1033.[13] They are composed of three regular α-amino acids and one β-amino acid with a long lipophilic side chain borne on the β-carbon (Fig. 1). These cyclic peptides show antifungal activities against *Candida albicans* and *Cryptococcus neoformans*. Because of the unique structure and biological activity, several analogues and peptidomimetics were synthesized for structure–activity relationship (SAR) studies.[14] Ohta et al. have shown that a lipophilic side chain ranging from 9 to 11 carbons, a bulky lipophilic group (Val) and a basic site (Lys or Orn) are crucial for antifungal activity. The spatial arrangement of these three side chains depends on the conformation of the cyclopeptide. The replacement of the natural β-aminoacid by a cyclobutane β-aminoacid bearing a straight carbon chain is expected to confer conformational rigidity upon the macrocycle, and possibly facilitate the macrocyclization step of its synthesis. We decided to test the hypothesis of using an aminoalcohol derivative as a stable synthetic equivalent of a C2-substituted *cis*-cyclobutane β-aminoacid by conducting the multi-step synthesis of the rhodopeptin analogue **10** (Fig. 1).

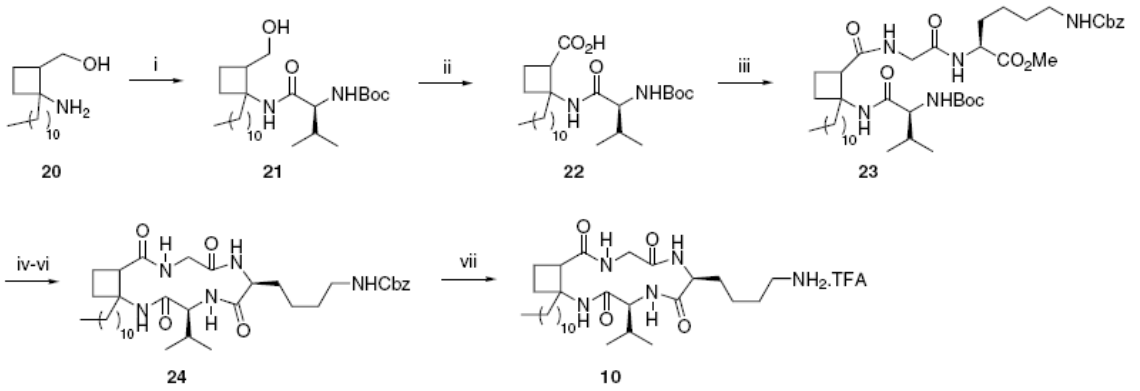
The appropriate β-aminoalcohol bearing a linear carbon chain ($C_{11}H_{23}$) **20** was prepared from 6-undecyluracil **14** (Scheme 3). This latter compound was synthesized in three steps from ethyl acetoacetate **11**, inspired by the literature procedures.[15] The [2+2] photocycloaddition protocol provided the *cis*-cyclobutane adduct **15** from 6-undecyluracil **14** and ethylene in a satisfying 86% yield.[16] Fears for the stability of **16** were confirmed: a retro-Mannich reaction occurs as previously mentioned. The treatment of heterocycle **15** with 0.5 M aqueous sodium hydroxide solution at reflux gave δ-ketoacid **17** directly and in quantitative yield.[17] As an aside, it is interesting to note that this whole sequence constitutes a double homologation of β-ketoester **12** to give δ-ketoacid **17**.

The inherent instability of aminoacid **16** was clearly demonstrated, so its surrogate, aminoalcohol **20**, was prepared in three steps. Bicyclic intermediate **15** was reduced using an excess of $NaBH_4$ in a THF/water mixture (due to the poor solubility of compound **15** in water alone) to give ureidoalcohol **18** in excellent yield (98%). The diazotization reaction of urea **18** was carried out in aqueous HCl/THF solution to provide cyclic carbamate **19** with a yield of 77%. Finally, aminoalcohol **20** was obtained by treatment of compound **19** with base (93% yield).[18]

Racemic compound **20** was coupled with Boc-L-valine using EDC/HOBt to provide compound **21** in 61% yield, without recourse to primary alcohol protection (Scheme 4). This compound was obtained as a mixture of diastereoisomers, which unfortunately could not be separated by chromatography. With the lone pair on the nitrogen effectively deactivated due to its participation in the peptide bond, subsequent oxidation of the primary alcohol of **21** should give a relatively stable dipeptide derivative of **16**. We selected $NaIO_4$/cat. $RuCl_3$ as the oxidation system, since it had already been shown to facilitate the formation of peptides containing 2,3-methanoaspartic acid.[19] In the event, dipeptide acid **22** was obtained smoothly; no retro-Mannich type ring-opening reactivity of this product was observed. Coupling of **22** with a Gly-Lys derivative using EDC/HOBt provided tetrapeptide **23** (31% yield in 2 steps from compound **21**), again as a diastereoisomeric mixture. Methyl ester



Scheme 3. Reagents and conditions: (i) NaH, BuLi, $C_{10}H_{21}Br$, THF, 0 °C; (ii) thiourea, EtONa, EtOH, reflux; (iii) $ClCH_2CO_2H$, H_2O, reflux, then concd HCl, reflux, 33% (3 steps); (iv) hν, ethylene, acetone, rt, 86%; (v) NaOH, 0.5 M, 100 °C, 100%; (vi) $NaBH_4$, H_2O/THF 1:1, rt, 98%; (vii) $NaNO_2$, HCl 3.5 M, THF, rt, 77%; (viii) NaOH, EtOH/H_2O, reflux, 93%.



Scheme 4. Reagents and conditions: (i) Boc-Val-OH, EDC, HOBt, CH_2Cl_2, rt, 61%; (ii) cat. $RuCl_3$, $NaIO_4$, $CCl_4/CH_3CN/H_2O$; (iii) H-Gly-N^{ε}-Cbz-Lys-OMe·TFA salt, Et_3N, EDC, HOBt, CH_2Cl_2, rt, 31% (2 steps); (iv) LiOH, H_2O/THF/CH_3OH, 0 °C, then rt, 99%; (v) TFA, CH_2Cl_2, 0 °C, then rt, 98%; (vi) DPPA, $NaHCO_3$, DMF, 5 °C, then rt, 71%; (vii) Pd/C 10%, TFA, CH_2Cl_2, 100%.

hydrolysis and Boc-group removal gave an intermediate tetrapeptide, which was macrocyclized using DPPA to the cyclic tetrapeptide **24** with a yield of 69% for three steps.[20] This is the first macrolactamization between the Val and Lys residues of a rhodopeptin analogue, and the hairpin-like turn imposed by the *cis*-cyclobutane β-aminoacid component[4c] probably facilitates the reaction. Quantitative removal of the Cbz group by hydrogenolysis in the presence of palladium on carbon in trifluoroacetic acid/dichloromethane media furnished the target rhodopeptin analogue **10** as its TFA salt.[21]

In conclusion, this initial study shows that an inherently unstable C2-substituted *cis*-cyclobutane β-aminoacid can be incorporated into a stable peptide, through use of its stable aminoalcohol surrogate. On this premise, we are currently developing access to enantiomerically pure rhodopeptin analogues for conformational studies and determination of biological activity.

Acknowledgments

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12. Compound **9**: Yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (s, 3H), 1.47 (m, 1H), 1.76 (m, 1H), 1.86 (m, 2H), 2.16 (m, 1H), 2.98 (ls, 3H), 3.68 (dd, $J = 11.4$, 4.6 Hz, 1H), 3.77 (dd, $J = 11.4$, 8.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.5 (CH_2), 29.7 (CH_3), 35.0 (CH_2), 45.7 (CH), 54.4 (C), 63.4 (CH_2). HRMS (ES^+): *m/z* calcd for $C_6H_{14}NO$ $[MH]^+$: 116.1075; found: 116.1084.
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16. Compound **15**: White powder, mp 132–133 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, $J = 7.2$ Hz, 3H), 1.30 (m, 18H), 1.66 (m, 2H), 2.17 (m, 2H), 2.30 (m, 1H), 2.39 (m, 1H), 3.02 (dd, $J = 7.0$, 9.4 Hz, 1H), 5.66 (s, 1H), 7.92 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1 (CH_3), 21.7 (CH_2), 22.7 (CH_2), 29.3 ($2CH_2$), 29.5 ($3CH_2$), 29.6 ($2CH_2$), 31.9 (CH_2), 34.1 (CH_2), 40.3 (CH_2), 40.7 (CH), 57.0 (C), 152.7 (C), 165.1 (C). ESMS *m/z* 317 $[MNa^+]$. Elemental analysis calcd for $C_{17}H_{30}N_2O_2$: C, 69.35; H, 10.27; N, 9.51. Found: C, 69.71; H, 10.27; N, 9.53.
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18. Compound **20**: Yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, $J = 7.0$ Hz, 3H), 1.25 (m, 18H), 1.51 (m, 2H), 1.63 (m, 1H), 1.72–1.85 (m, 2H), 1.94 (m, 1H), 2.19 (m, 1H), 2.53 (ls, 3H), 3.76 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9 (CH_3), 15.7 (CH_2), 22.5 (CH_2), 23.2 (CH_2), 29.2 (CH_2), 29.5 ($4CH_2$), 29.9 (CH_2), 31.7 (CH_2), 33.3 (CH_2), 42.7 (CH_2), 44.1 (CH), 57.0 (C), 63.2 (CH_2). HRMS (ES^+): *m/z* calcd for $C_{16}H_{34}NO$ $[MH]^+$: 256.2640; found: 256.2641.
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20. Analytical HPLC analysis of cyclic tetrapeptide **24** gave insufficient base-line separation, precluding isolation by preparative HPLC. Column Acclaim™ 120 C18 3 µm (2.1 × 100 mm); mobile phase CH_3CN–H_2O 70/30 + HCO_2H 1‰; temperature 30 °C; flow rate 0.25 mL/min; UV detection at 210 nm. t_R: 11.04 and 11.65 min.
21. Compound **10**: White solid, mp 117–119 °C, ^{13}C NMR (100 MHz, CD_3OD) δ 14.4 (CH_3), 17.4 (CH_3 Val), 18.0 (CH_2), 19.1 (CH_3 Val), 19.5 (CH_2), 20.0, 20.3, 23.7, 24.0, 24.1, 24.6, 24.7, 27.9, 30.5, 30.7, 30.7, 30.9, 31.2, 31.6 (CH Val), 31.9, 32.2, 33.1, 39.0, 40.4 (CH_2 Lys), 40.6, 44.2 (CH_2 Gly), 45.3 (CH_2 Gly), 50.0 (CH), 50.3 (CH), 57.7 (CH Lys), 57.9 (CH Lys), 59.1 (CH Val), 60.3 (C), 61.4 (CH Val), 62.5 (C), 171.6, 172.3, 172.6, 173.7, 174.4, 174.6, 175.5, 176.0. HRMS (ES^+): *m/z* calcd for $C_{29}H_{54}N_5O_4$ $[MH]^+$: 536.4176; found: 536.4180. Analytical HPLC: Column Acclaim™ 120 C18 3 µm (2.1 × 100 mm); mobile phase CH_3CN–H_2O 45/55 + HCO_2H 0.5‰; temperature 30 °C; flow rate 0.25 mL/min; UV detection at 210 nm. Single peak observed, t_R: 12.81 min.