Stereoselective Synthesis of a Functionalized 2-Oxo-1-azabicyclo[5.3.0]alkane as a Potential Scaffold for Targeted Chemotherapy Strategies

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Abstract: A stereoselective synthesis of functionalized 2-oxo-1-azabicyclo[5.3.0]alkane is presented. Key events of the synthetic sequence were the stereoselective propenylation of an N-acyliminium ion and a ring-closing metathesis reaction forming a 7-membered lactam.

Key words: metathesis, ring closure, lactams, bicyclic compounds, asymmetric synthesis, peptides

Many physiological processes including cell activation, migration, proliferation and differentiation require direct contact between cells and the extracellular matrix. These interactions are mediated through several different families of CAMs (cell adhesion molecules) including the selectins, the integrins, the cadherins and the immunoglobulins. In particular, integrins are a/β heterodimeric cell surface receptors which play a major role in cell–cell and cell–matrix adhesive interactions and represent the best opportunity of targeting small-molecule antagonists for both therapeutic and diagnostic use in various key diseases. Bicyclo[x.y.0]alkanes have served as scaffolds for the synthesis of integrin antagonists. In particular, we have found that the 2-oxo-1-azabicyclo[5.3.0]alkane 1 when incorporated in a cyclic RGD (Arg-Gly-Asp) peptide appears to force the two pharmacophoric groups of Asp and Arg to adopt the correct disposition to interact with the receptors (Figure 1).

Compound 3 has shown to be highly active and selective toward the α,β integrin which are expressed on the surface of a variety of cell types and are implicated in many pathological processes, such as tumor metastasis, angiogenesis, and osteoporosis. Therefore, 3 could be a potential antitumor drug, and is currently under study in various animal models.

Since the α,β integrin is overexpressed by many tumors, ST1646 could also be seen as a tumor-homing peptide and, as such, it could be used to improve the therapeutic index of other cancer chemotherapeutics by selective cell targeting. Thus, our recent efforts have been directed toward the synthesis of functionalised bicyclo[5.3.0]alkane scaffolds bearing appropriate side-chains that can be used to conjugate the most frequently used anticancer drugs to the integrin binders. Although many reports on the synthesis of bicyclic lactams can be found in the literature, only a few describe the preparation of azabicyclo[x.y.0]alkanes bearing functionalised side-chains. In the course of our studies on peptide secondary structure mimics we have already synthesized a functionalised 7,5-fused bicyclic lactam using a Horner–Emmons based strategy. However, the number of steps of this approach and the importance of these compounds forced us to investigate new synthetic routes. We now report a stereoselective synthesis based on ring closing metathesis of a 7,5-trans-fused bicyclic lactam with a suitably functionalised side-chain.

The starting material for the synthesis was the known 4-allyl pyroglutamic ester 4 (Scheme 1) which, after protecting group manipulation, was converted to the corresponding alcohol 5 via ozonolysis followed by in situ NaBH 4 reduction. Hydroxy group protection as the silyl ether (thexyldimethylsilyl chloride, imidazole in DME) followed by N-Boc nitrogen protection using standard conditions afforded the corresponding protected pyroglutamic acid 7 in 92% yield over the two steps. The order of the synthetic sequence was found to be crucial. If the ozonolysis and NaBH 4 reduction were performed starting from the N-Boc-protected pyroglutamate 8, an irreversible ‘ring switching’ to the lactone 9 was observed (Scheme 1).

Selective reduction of the imide 7 to the hemiaminal 11 (Scheme 2) was best performed with lithium triethylborohydride at –78 °C (99%). According to ample literature precedent and to our own experience on similar substrates, the hemiaminal 11 was first converted to the corresponding C5-methoxy derivative 12 by PPTS in MeOH (95%) and then subjected to boron trifluoride mediated 1-
propenyl cuprate addition as described by McClure et al. However, the main product observed after cuprate addition was arising by intramolecular nucleophilic attack of the hydroxy group on the intermediate hemiaminal. To avoid oxygen deprotection in the reaction conditions, the protecting group was changed from silyl ether to acetate. The propenyl cuprate addition occurred smoothly on the acetate to yield the 5-propenyl proline, as the only isomer. The product configuration was established by NOE experiments. Selective removal of the tert-butoxycarbonyl protecting group from with HClO⁴ in t-BuOAc gave the desired amine (79%).

The coupling of this hindered amine to a racemic mixture of N-Cbz allyl glycine proceeded with complete kinetic resolution and afforded as the only isomer in 64% yield using 3.3 equiv of acyl fluoride or the mixed anhydride preactivation methods. The configuration of the amino acid moiety in was not established at this point but was determined by NOE experiment on the final target 17.

Thus the stage was set for the RCM reaction of the dienyl precursor 16. The reaction proceeded in 16 h in refluxing CH₂Cl₂ in the presence of 10% of the Grubbs first generation catalyst to give the functionalized bicyclic scaffold in 83% yield. The reduction of the double bond was achieved by NaBH₄-NiCl₂ in MeOH (80%).

In summary, we have reported a convenient synthetic route for the preparation of a substituted 2-oxo-1-aza[5.3.0]bicycloalkane based on RCM cyclization. This bicyclic lactam could be used as scaffold for the synthesis of a cyclopentapeptide containing the RGD sequence for targeted chemotherapy strategies.

1H and 13C NMR spectra were recorded in CDCl₃ solution as indicated, at 200 (or 300, 400) and 50.3 MHz, respectively. The chemical shift values are given in ppm and the coupling constants J in Hz. Optical rotation data were obtained with a Perkin–Elmer model 241 polarimeter. TLC was carried out using Merck precoated silica gel F-254 plates. Flash chromatography was carried out using Macherey-Nagel silica gel 60, 230–400 mesh. Solvents were dried according to standard procedures, and reactions requiring anhyd conditions were performed under nitrogen. Petroleum ether refers to the fraction with bp 40–60 °C. Solutions containing the final products were dried (Na₂SO₄), filtered, and concentrated under reduced pressure.
pressure using a rotary evaporator. Elemental analyses were performed by the staff of the microanalytical laboratory in our department. The (Z)-1-lithio-1-propene was prepared from cis-1-bromo-1-propene (Aldrich) and lithium powder (high sodium, Aldrich) in Et$_2$O at 25 °C under an argon atmosphere in ca. 65% yield (based on the bromide). Concentrations of the nearly colorless ethereal solutions of the alkenyllithium were typically ca. 0.7 M as determined by titration. Abbreviations: DMAP, 4-(dimethylamino)pyridine; TBAF tetrabutylammonium fluoride; DMF, N,N-dimethylformamide; THF tetrahydrofuran; Boc$_2$O diborane; TSCl, thexyldimethylsilyl chloride; PPTS, pyridinium para-tolensulfonate; NMM, N-methylmorpholine.

(2S,4R)-4-Hydroxethyl-5-oxopyrrolidine-2-carboxylic Acid tert-Butyl Ester (5)

A stirred solution of 4 (2.1 g, 9.32 mmol) in MeOH (93 mL) was cooled to –78 °C, whereupon Et$_3$N was bubbled through it (flow rate 30 L/h). After 1.5 h, NaBH$_4$ was added through the reaction mixture in order to eliminate the excess of O$_3$. The organic layers were dissolved in EtOAc and washed with brine. The organic phase was evaporated under reduced pressure and the residue was re-dissolved in EtOAc and washed with brine. The organic phases were dried (Na$_2$SO$_4$) and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (EtOAc–petroleum ether, 2:8) to yield 5.

Yield: 1.04 g (94%); yellow oil.

(2S,4R)-4-[2-(Dimethyl-(1,1,2-trimethylpropyl)silanyloxy)ethyl]-5-hydroxypyrrrolidine-1,2-dicarboxylic Acid tert-Butyl Ester (11)

To a solution of 7 (0.660 g, 1.40 mmol) in anhyd THF (14 mL), LiEt$_2$BH (1 mL in THF; 1.68 mL was added at –78 °C and the solution was stirred for 50 min, then sat. aq NH$_4$Cl solution (15 mL) was added. The aq phase was extracted with EtOAc, the combined organic layers were dried (Na$_2$SO$_4$), filtered and the solvent was removed under reduced pressure. The crude product, as a yellowish oil, was submitted to the next reaction without further purification.

1H NMR (200 MHz, CDCl$_3$); δ = 0.12 [s, 6 H, Si(C$_2$H$_5$)$_2$], 0.86 (m, 12 H, CH$_3$), 1.45 [s, 9 H, C(CH$_3$)$_3$], 1.50–2.20 (m, 16 H), 3.60 (m, 2 H, CH$_2$OTDS), 4.15 (m, 1 H, CHCOO-t-Bu), 5.25, 5.32 (2 m, 1 H, CHO$_2$).

MS (FAB): m/z calcd for C$_{25}$H$_{49}$NO$_6$Si: 487.33; found: 488.

Anal. Calcd for C$_{25}$H$_{49}$NO$_6$Si: C, 59.20; H, 9.06; N, 4.05.

(2S,4R)-4-[2-(Dimethyl-(1,1,2-trimethylpropyl)silanyloxy)ethyl]-5-methoxypyrrrolidine-1,2-dicarboxylic Acid tert-Butyl Ester (12)

To a solution of 11 (0.598 g, 1.26 mmol) in MeOH (14 mL), PPTS (0.453 g, 1.80 mmol) was added. After 12 h, the solvent was evaporated and to the residue was added a phosphite buffer solution (10 mL). The aq phase was extracted with CH$_2$Cl$_2$, the combined organic layers were dried (Na$_2$SO$_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (EtOAc–petroleum ether, 1:9) to yield 12.

Yield: 0.580 g (94%); colorless oil.

1H NMR (200 MHz, CDCl$_3$); δ = 0.12 [s, 6 H, Si(C$_2$H$_5$)$_2$], 0.86 (m, 12 H, CH$_3$), 1.45 [s, 9 H, C(CH$_3$)$_3$], 1.50–2.35 (m, 15 H), 3.40 (s, 3 H, OCH$_3$), 3.60 (m, 2 H, CH$_2$OTDS), 4.10 (m, 1 H, CHCOO-t-Bu), 5.01, 5.10 (2 m, 1 H, CHO$_2$).

MS (FAB): m/z calcd for C$_{25}$H$_{49}$NO$_6$Si: 487.33; found: 488.

Anal. Calcd for C$_{25}$H$_{49}$NO$_6$Si: C, 61.56; H, 10.15; N, 2.87.

Found: C, 61.68; H, 10.15; N, 2.87.
To a solution of the alcohol (0.102 g, 0.30 mmol) in THF (3.0 mL) were added, at 0 °C, Et 3 N (0.049 mL, 0.32 mmol) and acetyl chloride (0.023 mL, 0.35 mmol). After 30 min, the mixture was warmed to r.t. and stirred for 4 h. After 10 min, a solution of CuBr·DMS (0.114 g, 0.56 mmol) was then added slowly. After 10 min, a solution of (EtOAc–petroleum ether, 35:65) to yield 15. Yield: 0.064 g (64%); yellowish oil; [α] 235 0° –9.5 (c 1.0, CHCl 3 ).

1H NMR (400 MHz, CDCl 3 ); δ = 1.48 [s, 9 H, C(CH 3 ) 3 ], 1.50 (m, 1 H, HCHCDCH 2 CH 2 OAc), 1.58 (m, 1 H, HCHCDCH 2 OAc), 1.60 (m, 1 H, HCHCDCH 2 OAc), 1.70 (m, 3 H, CH=CH 2 ), 1.90 (m, 2 H, HCDCHOAc, HCHCDCH 2 OAc), 2.05 (3, s, 3 H OCOCH 3 ), 2.40 (m, 1 H, HCDCHOAc, HCHCDCH 2 OAc), 2.50 (3, s, 3 H OCOCH 3 ), 2.67 (m, 1 H, HCHCDCH 2 OAc), 2.70 (H, HCHCDCH 2 OAc), 3.40 (m, 2 H, CHCOOBu-), 4.00 (m, 2 H, CHCOOBu-), 4.32 (m, 1 H, CHCOOBu-), 5.10 (m, 2 H, CHCOOBu-). Anal. Calcd for C 16 H 27 NO 4 : 297.19; found: 298.

To a solution of (EtOAc–petroleum ether, 35:65) to yield 16. Yield: 0.020 g (61%); yellowish oil; [α] 235 0° –9.5 (c 1.0, CHCl 3 ).

1H NMR (400 MHz, CDCl 3 ); δ = 1.48 [s, 9 H, C(CH 3 ) 3 ], 1.50 (m, 1 H, HCHCDCH 2 CH 2 OAc), 1.58 (m, 1 H, HCHCDCH 2 OAc), 1.60 (m, 1 H, HCHCDCH 2 OAc), 1.70 (m, 3 H, CH=CH 2 ), 1.90 (m, 2 H, HCDCHOAc, HCHCDCH 2 OAc), 2.05 (3, s, 3 H OCOCH 3 ), 2.40 (m, 1 H, HCDCHOAc, HCHCDCH 2 OAc), 2.50 (3, s, 3 H OCOCH 3 ), 2.67 (m, 1 H, HCHCDCH 2 OAc), 2.70 (H, HCHCDCH 2 OAc), 3.40 (m, 2 H, CHCOOBu-), 4.00 (m, 2 H, CHCOOBu-), 4.32 (m, 1 H, CHCOOBu-), 5.10 (m, 2 H, CHCOOBu-). Anal. Calcd for C 16 H 27 NO 4 : 297.19; found: 298.
Anal. Calcd for C_{26}H_{34}N_{2}O_{7}: C, 64.18; H, 7.04; N, 5.76. Found C, 64.30; H, 7.05; N, 5.75.

To a solution of the cyclized compound (0.0249 g, 0.051 mmol) in MeOH (0.5 mL) were added NaClO_{4}·6 H_{2}O (0.012 g, 0.051 mmol) and NaBH_{4} (0.01 g, 0.256 mmol). After 1 h, silica gel was added and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (EtOAc–petroleum ether, 4:6) to yield 17.

Yield: 0.021 g (80%); yellowish oil; [α]_{D}^{20} = 24.9 (c 1.0, CHCl_{3}).

{\textsuperscript{1}H} NMR (400 MHz, CDCl_{3}): δ = 1.30 (m, 2 H, HCH=CHCH_{2}CH_{2}OAc), 4.28 (m, 1 H, CCH_{2}CH_{2}OAc), 4.11 (m, 2 H, HCHCOO-t-Bu), 6.28 (m, 1 H, NH), 7.35 (m, 5 H, aromatics).

{\textsuperscript{13}C} NMR (50.3 MHz, CDCl_{3}): δ = 171.2, 171.1, 170.7, 155.5, 136.7, 128.6, 128.1, 81.7, 66.5, 60.5, 62.6, 61.5, 55.0, 43.8, 35.1, 33.0, 32.5, 31.8, 28.1, 27.3, 21.1.

MS (FAB): m/z calc for C_{26}H_{36}N_{2}O_{7}: 488.25; found: 489.

Anal. Calcd for C_{26}H_{36}N_{2}O_{7}: 488.25; found: 489.

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