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Design and Synthesis of a Protein:Farnesyltransferase Inhibitor based on Sugar Amino Acids

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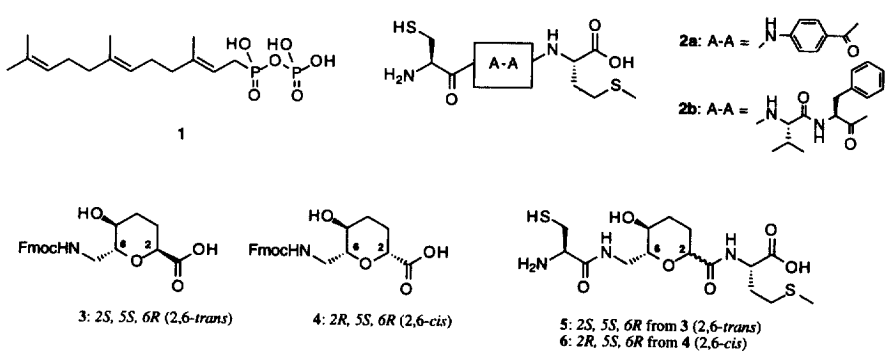
Abstract: The synthesis of four partially deoxygenated gluconic amino acids from fully acetylated D-glucal is described. Replacement of the central AA dipeptide in the CAAX tetrapeptide corresponding to the C-terminus of K-Ras p21 by one of these sugar amino acid building blocks led to a novel protein:farnesyl transferase inhibitor.

Key words: sugar amino acid, tri-*O*-acetyl-glucal, Ferrier rearrangement, protein:farnesyl transferase inhibitors.

The small GTP-binding proteins of the Ras-family play a crucial role in the transduction of growth and differentiation signals from receptor tyrosine kinases to the cell nucleus, resulting in the switching on of an intracellular phosphorylation cascade.[1] An essential prerequisite for the function of a Ras protein is its association with the plasma membrane. The latter event is initiated by post-translational farnesylation of the cysteine unit of the so-called CAAX box (C, cysteine; A, any aliphatic amino acid; X, serine or methionine) in the pre-Ras protein by the enzyme protein:farnesyl transferase (PFT).[2] Oncogenic Ras proteins are locked in the activated GTP-bound state which leads to a continuously switched on phosphorylation cascade. The development of effective PFT inhibitors would be of great importance in combating colon and pancreatic carcinomas.[3] Apart from natural products, several inhibitors based on farnesyl pyrophosphate have been published.[4] For instance, our laboratory revealed that the farnesyl pyrophosphate analogue **1** is a potent inhibitor.[4a] On the other hand, mimetic replacement of one or two



A's in the CAAX motif of the natural K-Ras p21 substrate is a very rewarding alternative.[5] For example, potent PFT inhibitors were obtained by replacing the AA moiety by a rigid spacer (*e.g.* 4-aminobenzoic acid as in **2a**)[5a] or a more flexible dipeptide (*e.g.* Val-Phe as in **2b**),[5b] indicating that the AA portion is

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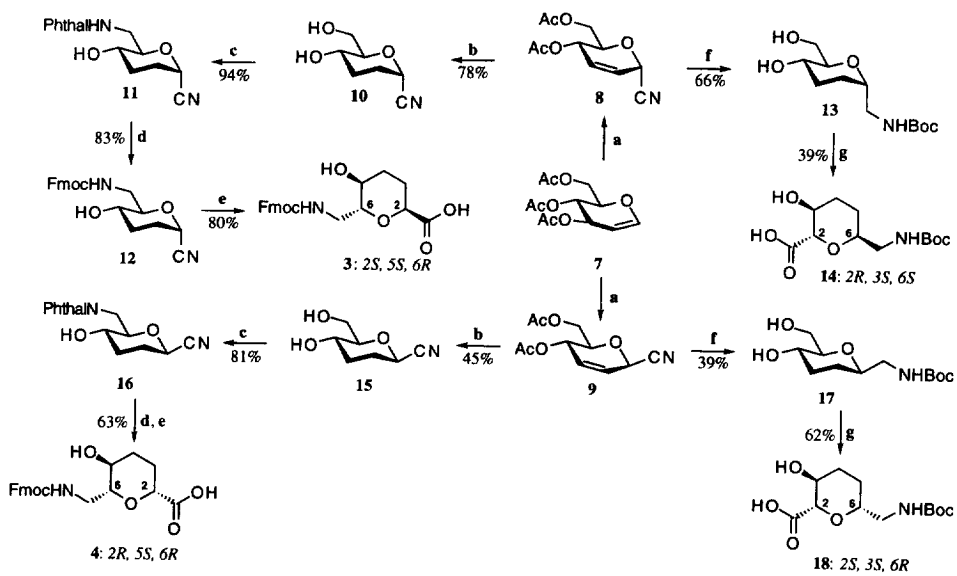
amenable to different conformations depending on the binding environment.[6] In order to get a better insight into the bioactive conformation of CAAX peptidomimetics, it would be of interest to explore the effect of replacing AA by a conformationally restricted dipeptide isosteric pyranoid sugar amino acid (SAA). Herein, we report the synthesis of the "2,6-*trans*" and "2,6-*cis*"-pyranoid SAAs **3** and **4** as well as their subsequent incorporation into CAAM in its AA position resulting in peptidomimetic analogues **5** and **6**.

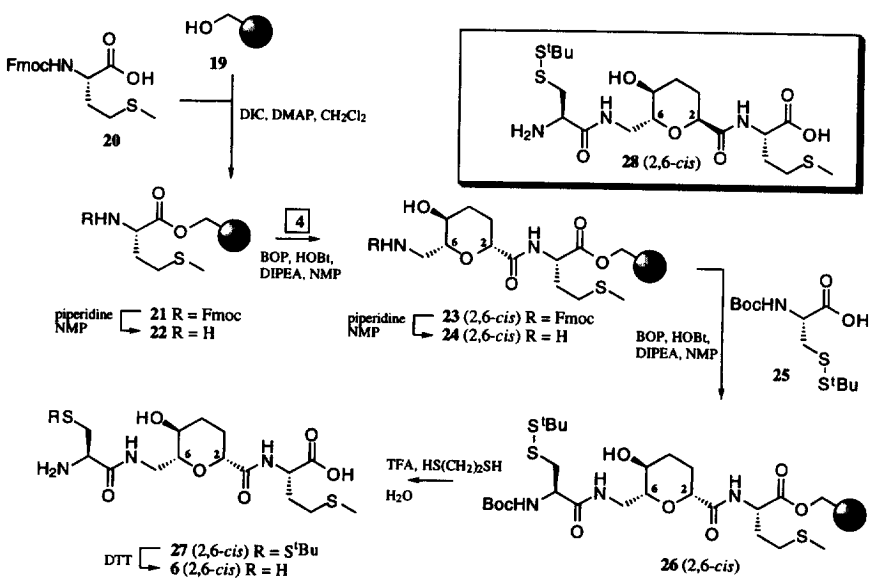
The first step in the synthesis of both glucopyranoid amino sugars **3** and **4** (Scheme 1) is a Ferrier rearrangement of 3,4,6-tri-*O*-acetyl-D-glucal (**7**) with trimethylsilyl cyanide under the influence of catalytic $BF_3.OEt_2$ in the solvent dichloromethane leading to the isolation of the individual anomeric cyanides **8**α and **9**β in a yield of 56 and 42%, respectively.[7] Hydrogenation of the double bond in the major isomer **8** gave, after deacetylation, diol **10** in an overall yield of 78%.[8] Substitution of the primary hydroxyl group of **10** with phthalimide under Mitsunobu conditions proceeded in a regioselective manner. Hydrazinolyzis of the phthaloyl group in **11** and subsequent reaction of the newly generated amine function with FmocCl gave, after acid hydrolysis of the partially protected cyanide derivative **12**, the requisite "2,6-*trans*"-SAA building block **3**[8] in an overall yield of 49% (based on **8**). In a similar fashion, the minor isomer **9** could be readily transformed into the "2,6-*cis*"-SAA building unit **4**[8] (overall yield 23% based on **8**).[9] At this stage, it is of interest to note that either **8** or **9** are also suitable starting compounds for the synthesis of an additional set of the diastereoisomeric SAA building units **14** and **18**. Thus, hydrogenation of **8** in the presence of acid and reaction of the resulting amine function with di-*tert*-butyl dicarbonate (Boc_2O) gave, after deacetylation, the diol **13**.[10] Regioselective TEMPO oxidation[11] of the primary hydroxyl group in **13** afforded the "2,6-*trans*"-SAA unit **14** in an overall yield of 26% (based on **8**). Similarly, transformation of **9** *via* the four-step sequence of reactions gave the "2,6-*cis*" SAA unit **18** in a comparable yield.



Reagents and conditions: **a**) TMSCN, $BF_3.OEt_2$, CH_2Cl_2 (56% **8**, 42% **9**), **b**) H_2 (1 atm.), Pd/C, EtOAc, then NaOMe, MeOH, **c**) phthalimide (1.4 eq.), PPh_3 (1.2 eq.), DEAD (1.2 eq.), THF, reflux, **d**) NH_2NH_2 (4 eq.), MeOH, then FmocCl, DIPEA, DMF, **e**) HCl (conc.)/dioxane 1/1, reflux, **f**) H_2 (3 atm.), Pd/C EtOAc/*i*-PrOH/EtOH 2/1/1, HCl (1.1 eq.), then Boc_2O, Et_3N, DMF, then NaOMe, MeOH, **g**) tetramethylpiperidinooxy free radical (TEMPO) (cat.), KBr, NaOCl, NaOH, H_2O.

Scheme 1

The solid phase synthesis of the target peptidomimetics **5** and **6** commences, as outlined in Scheme 2, with the loading of the Fmoc-protected methionine derivative **20** to the Wang solid support **19** under the influence of *N,N*-diisopropylcarbodiimide (DIC) and DMAP. Removal of the Fmoc group in **21** with piperidine in the solvent *N*-methyl-pyrrolidinone (NMP), followed by elongation of **22** with the "2,6-*cis*" monomer **4** in the presence of BOP/HOBt and *N,N*-diisopropylethylmine (DIPEA), led to the formation of the immobilized dimer **23**. Extension of the Fmoc-deprotected dimer **24** with the *S*-*tert*-butyl and *N*-Boc protected cysteine derivative **25** following the same coupling procedure gave the immobilized peptidomimetic **26**. Treament of **26** with TFA in the presence of the scavenger 1,2-ethanediol resulted, after purification by reversed phase HPLC, in the isolation of the homogeneous "2,6-*cis*" peptide **27**[8] in a yield of 15%. Similarly, use of the carbopeptoid **3**, instead of **4**, led to the isolation of the corresponding "2,6-*trans*"-peptidomimetic derivative **28**[8] in 20% yield. In this respect, it is of interest to note that the solution phase synthesis of the analogous methyl esters starting from methionine methyl ester and using the same coupling reagents (i.e. BOP, HOBt, DIPEA) proceeded in an overall yield of 60%. Deprotection of the *N*-terminal cysteine moiety in **27** and **28** could be easily effected, as gauched by reversed phase HPLC, with excess dithiothreitol (DTT)[12] in a TRIS.HCl buffer (pH 7.5) to give, after purification, the target compounds **5** and **6** in a near quatitative yield.



Scheme 2

The IC_{50} values determined[13] of both CAAX analogs revealed that the "2,6-*cis*"-isomer **6** was a more effective inhibitor (IC_{50} of 214 μM) of bovine PFT than the "2,6-*trans*"-isomer **5** (IC_{50} of 764 μM). Nonetheless, the inhibitory effect of **6** is not as strong as those reported for the inhibitors **2a** and **2b** and other CAAX-based inhibitors.[5c] However, the distinct inhibitory effect of **6**, resulting from the replacement of the AA portion in the CAAX box by a randomly chosen gluconic amino acid, forms a solid base in pursuing the design and synthesis of more powerful inhibitors via a combinatorial approach. Thus, apart from applying different sugar amino acids, the diversity of this approach can be extended further by taking into consideration that the number of the protected/unprotected hydroxyl group(s) of the furanoid/pyranoid rings[14] can be used to influence the hydrophobic/hydrophilic nature of the peptides. The results of this ongoing study will be reported in due course.

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[8] All new compounds were obtained in an analytically pure form and characterized by spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR, MS). Selected data: **3**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.76-7.26 (m, 8H), 5.38 (dd, 1H, *J* 7.4 and 5.3 Hz), 4.53 (dd, 1H, *J* 10.7 and 6.7 Hz), 4.44-4.36 (m, 2H), 4.18 (t, 1H, *J* 6.5 Hz), 3.76-3.59 (m, 2H), 3.27-3.13 (m, 2H), 2.24 (bd, 1H, *J* 13.3 Hz), 2.00-1.95 (m, 1H), 1.84-1.73 (m, 1H), 1.52-1.38 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 174.9, 158.3, 143.7, 143.5, 141.2, 127.7, 127.0, 124.9, 119.9, 77.2, 72.0, 65.9, 65.8, 47.1, 41.6, 27.8, 25.9. MS: *m/z* 420.1 $(M+Na)^+$. **4**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.78-7.29 (m, 8H), 5.87 (dd, 1H, *J* 7.9 and 4.9 Hz), 4.52 (dd, 1H, *J* 6.7 and 10.6 Hz), 4.40 (m, 1H), 4.19 (t, 1H, *J* 6.6 Hz), 3.95 (bd, 1H, *J* 10.5 Hz), 3.76-3.69 (m, 1H), 3.25-3.17 (m, 3H), 2.12-2.05 (m, 2H), 1.58-1.44 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.3, 158.3, 143.6, 143.5, 141.2, 127.5, 126.9, 124.9, 124.8, 119.8, 81.0, 75.1, 66.4, 65.2, 47.1, 41.3, 30.7, 28.3. MS: *m/z* 420.2 $(M+Na)^+$. **27** (TFA salt): ^{1}H-NMR (600 MHz, $CDCl_3$): δ 4.47 (dd, 1H, *J* 4.3 and 8.3 Hz; $H_\alpha$met), 4.27-4.24 (m, 2H; $H_\alpha$cys, H_2), 3.84-3.81 (m, 1H; H_6), 3.67-3.62 (m, 2H; H_5, H_7), 3.38 (dd, 1H, *J* 3.6 and14.5 Hz; H_7), 3.21-3.13 (m, 2H; $H_\beta$cys), 2.58-2.48 (m, 2H; $H_\gamma$met), 2.21-2.14 (m, 1H; $H_\beta$met), 2.06 (s, 3H; SMe) 2.05-2.00 (m, 1H; $H_\beta$met), 1.97-1.94 (m, 2H; H_3, H_4), 1.83-1.78 (m, 2H; H_3, H_4), 1.63-1.58 (m, 1H; H_3, H_4), 1.28 (s, 9H; S^{t}Bu). LCMS: *m/z* 498.4 $[MH^+]$. **28** (TFA salt): ^{1}H-NMR (600 MHz, $CDCl_3$): δ 4.45 (dd, 1H, *J* 4.7 and 8.9 Hz; $H_\alpha$met), 4.23 (dd, 1H, *J* 6.3 Hz; $H_\alpha$cys), 3.98 (bd, 1H, *J* 9.4 Hz; H_2), 3.65 (dd, 1H, *J* 6.3 and 14.5 Hz; H_7), 3.48 (dd, 1H, *J* 2.0 and 14.5 Hz; H_7), 3.46-3.40 (m, 1H; H_5), 3.38-3.35 (m, 1H; H_6), 3.22 (dd, 1H, *J* 6.0 and 14.5 Hz; $H_\beta$cys), 3.15 (dd, 1H, *J* 6.6 and 14.5 Hz; $H_\beta$cys), 2.61-2.55 (m, 1H; $H_\gamma$met), 2.53-2.47 (m, 1H; $H_\gamma$met), 2.20-2.12 (m, 2H; $H_\beta$met, $H_{3/4}$), 2.06 (s, 3H; SMe), 2.04-1,97 (m, 2H; $H_\beta$met, $H_{3/4}$), 1.59-1.51 (m, 2H; $H_\beta$met, $H_{3/4}$), 1.28 (s, 9h; S^{t}Bu). LCMS: *m/z* 498.4 $[MH^+]$.

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