Pergamon

*Tetrahedron Letters*, Vol. 38, No. 32, pp. 5697-5700, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01249-5

# Combinatorial Chemistry of Piperidine Based Carbohydrate Mimics.

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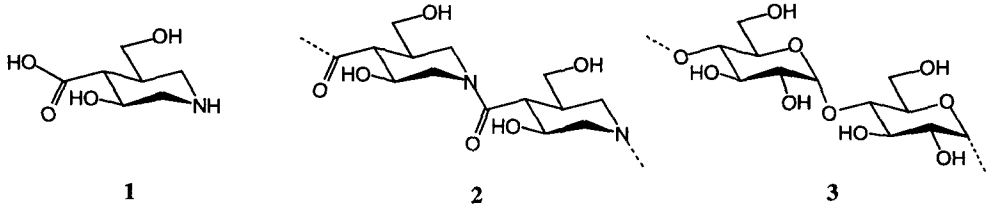
***Abstract:*** Piperidine carboxylic acids and 4-hydroxypiperidine-3-carboxylic acid, the latter obtained from bakers yeast reduction of the corresponding piperidone, were coupled in solid-phase synthesis to form simplified oligosaccharide analogues. A split-and-mix synthesis approach was used to create small combinatorial libraries which were characterised by LC-MS and screened as inhibitors of glycosidases.


Oligosaccharides constitute an interesting and innovative group of compounds to explore for bioactivity since these molecules may lead to new drugs, therapeutic treatments or diagnostic applications in a number of areas. Thus, the $sLe^X$ tetrasaccharide or analogues hereof may be used to treat inflammation,[1] while other oligosaccharides or analogues may be specific glycosidase inhibitors with the potential of treating diabetes,[2] AIDS[3] or cancer.[4] Therefore, the application of combinatorial chemistry to synthesise compounds of this class would be highly attractive,[5] but is hampered by the fact that solid-phase synthesis of oligosaccharides is still in its infancy.[6]

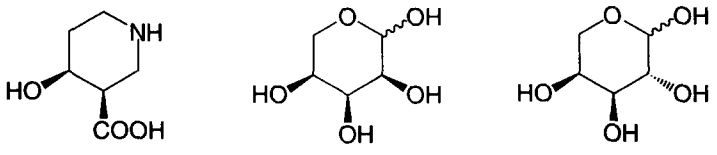
We have chosen to approach this problem by synthesising oligosaccharide analogues possesing an amide bond in place of the glycosidic linkage, which will allow exploitation of the powerful techniques of solid-phase peptide synthesis and combinatorial chemistry. The fundamental concept, as outlined below in Fig 1, employs various hydroxy- or hydroxymethyl substituted piperidine carboxylic acids, *e.g.* **1** as residues in a peptide **2** which have a high degree of resemblance with oligosaccharide **3**. Other peptide based carbohydrate mimetics have been reported recently,[7] however, in contrast to these reports our approach would lead to compounds bioisosteric with an oligosaccharide.




**1** **2** **3**

**Fig 1.** Structure of monomer and oligomer of oligosaccharide analogue.

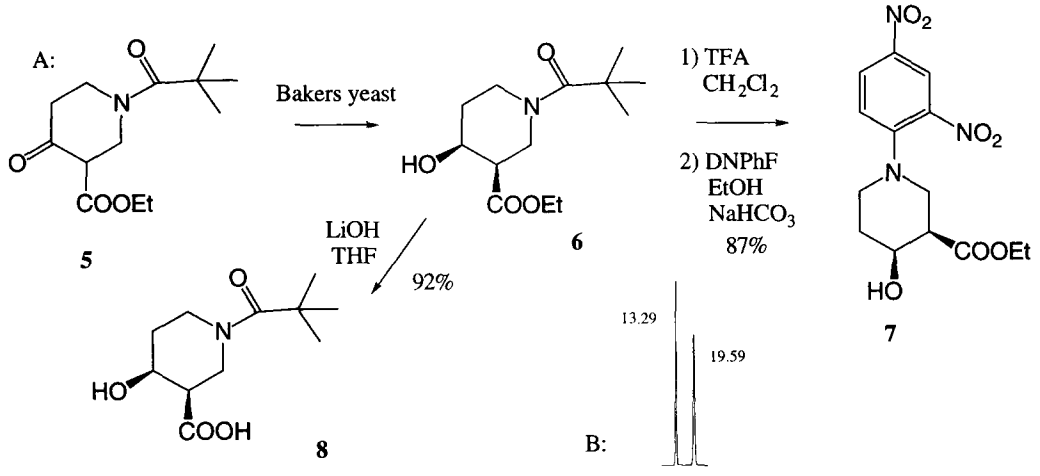
In this paper we report a solid-phase combinatorial synthesis of trisaccharide mimetics containing 4-hydroxypiperidine-3-carboxylic acid **4**. Compound **4** resembles L-ribose and L-arabinose while its enantiomer resembles the corresponding D-sugars.




4 L-ribose L-arabinose

**Fig 2.** Comparison of monomer **4** with pentoses.

Encouraged by the report that optically pure **6**, could be obtained in 78% yield, with high diastereoselectivity and enantioselectivity[8] by bakers yeast reduction of readily available ketone **5**,[9] we planned to obtain **4** by mild saponification of **6** (scheme 1). We were, however, unable to reproduce the results as described but instead made two important observations. First, when the bakers yeast reduction of **5** (Table 1) was carried out with fermenting bakers yeast (sucrose added) the alcohol **6** was obtained (m.p. and NMR as ref. 8) with complete diastereoselectivity but no enantioselectivity.[10] This was in contrast to other bakers yeast reductions of cyclic β-ketoesters, which usually give high enantioselectivity.[11] Varying the source or amount of yeast or time did not improve stereoselectivity. Secondly, when non-fermenting conditions were




**Scheme 1.** A: The synthetic scheme for the synthesis of **8**. B: Chiral HPLC of **7** (retention time in min).

used,[12] **6** was obtained with a rotation from +15° to +23° (in 2 experiments), which was close to the +25° previously described. However this material was found to have an enantiomeric excess of only 24% and thus much lower than the reported 93% e.e.[8]. The enantiomeric excess of the samples of **6** was conveniently measured by removal of the BOC-group from **6** with TFA, reaction of the resulting piperidine with Sanger's reagent[13] (2,4-dinitrofluorobenzene, DNPhF) to give **7** and analysis of **7** on chiral HPLC (Scheme 1B).[14]

Since optically pure **6** could not be obtained we employed the racemic **8** in our subsequent library construction. Hydrolysis of (±)-**6** with LiOH in THF gave **8** (mp. 140-144°C, MS(EI) m/z 246 (M+1), 146 (M-BOC)) in 92% yield.

A small combinatorial library of 27 members using 3 different monomers was made using the split-and-mix strategy. The monomers were N-*tert*-butoxycarbonyl-4-piperidinecarboxylic acid **9**, N-*tert*-butoxycarbonyl-R-pipecolic acid[15] **10** and the above described BOC-protected 4-hydroxypiperidine-3-carboxylic acid **8**. The monomers **9** and **10** were both BOC-protected according to a non-aqueous procedure[16].

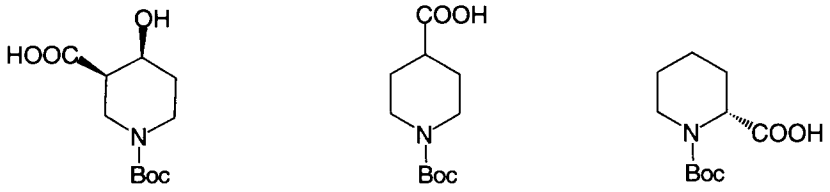
**Table 1**. Conversion of **5** to **6** using Commercial Bakers Yeast.

| Sucrose added | Time (days) | Yield **6** (recovered **5**) | $[\alpha]_D$ of **6** (e.e.) |
|---|---|---|---|
| + | 1 | 39% (28%) | + 0.2° (0%) |
| +* | 1 | 47% (27%) | + 0.4° |
| + | 3 | 34% (55%) | -2° |
| + | 5 | 23% (43%) | -4° |
| - | 1 | 39% (7%) | +15° (24%) |
| -* | 1 | 37% (33%) | +22 ° |

* Yeast obtained from Sigma.

The solid-phase synthesis was carried out on a MBHA-resin[17] using HATU[18] (*N*-[(dimethylamino)-*H*-1,2,3-triazolo[4,5-b]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate) as coupling reagent in DCM/DMF (1:1).[19,20]

The 3 sub-libraries of each 9 members thus obtained were cleaved from the resin using HF and extracted with TFA.[21] The different sub-libraries were analyzed on HPLC[22] and LC-MS[23] and tested for glycosidase inhibitory activity. Neither α-glucosidase (yeast), β-glucosidase (almonds), isomaltase (yeast), α-fucosidase (human placenta), β-mannosidase (snail), β-galactosidase (E. Coli) were inhibited significantly. Studies to test this library and larger libraries for bioactivity including enzyme inhibition and lectin binding is underway.




**8** **9** **10**

**Fig 3.** Building blocks in the combinatorial synthesis.

In this communication, we have reported the first example of a solid-phase combinatorial synthesis of an amine based carbohydrate mimetic. This approach has been found to be a viable way to prepare libraries of bioisosteric carbohydrate mimetics.

## ACKNOWLEDGEMENTS

We acknowledge financial support from the Danish National Science Research Council (SNF) grant nr. 9502986. We also thank B. O. Pedersen for Mass spectra and Dr. Per F. Nielsen, Novo Nordisk A/S, for LC-MS analysis.

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10. Typical procedure: bakers yeast (10 g) was dissolved in $H_2O$ (80 mL) at 30°C, and sucrose (15 g) was added. After 1 h at 30°C, compound **5** (1.71 g, 6.3 mmol) was added, and the reaction was kept at 25°C for 18 h. Sucrose (10 g) dissolved in $H_2O$ (25 mL) at 40°C was added, and the mixture was kept for 2 days at 25°C. Celite (1 g) was added, and the mixture was filtered. The filter was washed with $CHCl_3$ (3x20 mL), which was subsequently used to extract the filtrate. The combined $CHCl_3$ was dried, concentrated and subjected to flash-chromatography. Eluting with EtOAc-pentane 1:15 gave unreacted **5** (0.93 g, 54%), while eluting with EtOAc gave **6** (0.59 g, 34%, mp: 56-60°C, $[\alpha]_D$: -2 (*c* 0.75, $CH_2Cl_2$)).
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19. MBHA-resin (900 mg ) was split in three. Each portion was coupled for two hours with a monomer **8**, **9** or **10** (3eq.) using HATU (2.5 eq) and DIEA (6eq) in 3 ml of DMF/DCM (1:1). The monomer was prereacted with HATU and DIEA for 2 min. to avoid formation of byproducts (Gausepohl, H; Pieles, U; Frank, R. W. *Peptides, Chemistry and Biology,* **1992**, 523-524.) After reaction, the resin was washed with 3x2 ml DMF and 3x2 ml DCM. The three portions of resin were mixed, treated with 50% TFA/DCM (2x10min) to remove the BOC protecting group and split into three. The three fractions were reacted as described above with respectively **8**, **9** and **10**, washed, mixed, deprotected, split and reacted a last time with the tree monomers.
20. It is not possible to follow the reaction using the Kaiser-test, as this is inaccurate for secondary amines.
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22. HPLC was performed on a LiChrospher 100 RP-18 using a linear gradient of A (0.1% TFA/$H_2O$) and B (0.1% TFA/80% $CH_3CN/H_2O$) as mobile phase and a flow of 1 ml/min. The sublibraries were deprotected and reacted in DMF with Sanger´s reagent and DIEA. After cleavage of the resin, the HPLC were recorded at 390 nm.
23. Each of the sub-libraries were reacted with Sanger´s reagent and analyzed by LC-UV/MS. As monomer **9** and **10** have the same mass in total 4 different masses (516.23, 532.23, 548.22 and 564.22) were expected, and with 3 different masses in each sub-library. Within the chromatograms of the three individual sub-libraries, peaks having the expected masses were identified in all cases.


(*Received in UK* 16 *May* 1997; *revised* 19 *June* 1997; *accepted* 20 *June* 1997)