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Tetrahedron Letters
Tetrahedron Letters 47 (2006) 9203–9206

# An improved synthesis of morpholino-glycoamino acids

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Received 3 October 2006; revised 20 October 2006; accepted 26 October 2006

**Abstract**—The current synthesis of hybrid morpholino-glycoamino acids through double reductive amination is characterized by modest yields and lengthy reaction times. We propose an optimized procedure that results in improved yields and the shortest reaction times reported so far.


Morpholines are used extensively in organic synthesis as simple bases or as N-alkylating agents.[1] In recent years, morpholines have received considerable attention as glycomimetics—surrogates for monosaccharide moieties. For example, morpholine oligomers have been used as nucleic acid ribose surrogates with favourable antisense properties.[2] A disaccharide mimetic with a galactose moiety transformed to a hybrid morpholino-glycopeptide was found to be a better substrate for human milk fucosyltransferase than the native substrate.[3] Furthermore, morpholines have been used as glycomimetics in the synthesis of paromomycin analogues[4] and of *N*-substituted 1,5-dideoxy-1,5-imino-D-arabinitol 2,3,4-triphosphates from the parent α-trinositol.[5]

Hindsgaul and Du have developed a straightforward methodology for the synthesis of hybrid morpholino-glycoamino acids consisting of $NaIO_4$-mediated oxidation of glycopyranosides to dialdehydes, and subsequent double reductive amination to form functionalized morpholines with preserved stereochemistry.[3] The methodology is closely related to that reported by Acton et al.[6] Osborn and Clark later reported a modification of this synthesis.[7] A careful control of $NaIO_4$-mediated oxidation can lead to ring cleavage with or without extrusion of the glycopyranose C-atom at position 4, thus opening up the route to stereodefined morpholines and [1,4]-oxepanes.[7] A similar procedure for synthesizing morpholines, starting from modified glycofuranosides was reported by Grotenbreg et al.[8] We needed to develop an efficient synthesis of orthogonally protected, functionalized morpholino-glycoamino acids as versatile and 'drug-like' surrogates for *N*-acetylmuramic acid (Fig. 1). The latter is a constituent of bacterial cell walls and has been incorporated in a series of potent phosphinate inhibitors of the MurD enzyme, which are promising new antibacterial agents.[9]

The protected and functionalized morpholino-glycoamino acid should allow specific attachment of various substituents after deprotection. We prepared morpholine **5** based on the synthetic pathway of Hindsgaul and Du,[3]

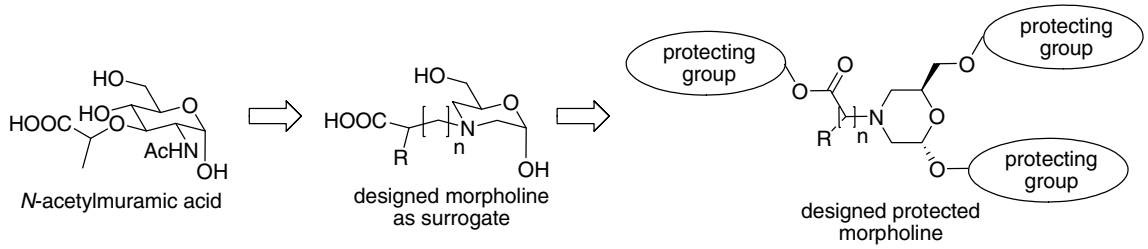


**Figure 1.**

*Keywords*: Morpholino-glycoamino acids; Glycomimetics; Reductive amination; Microwave-assisted synthesis.
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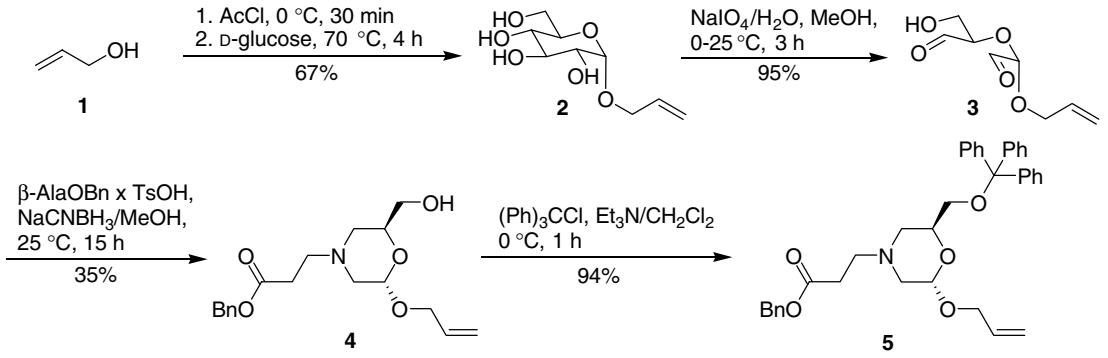
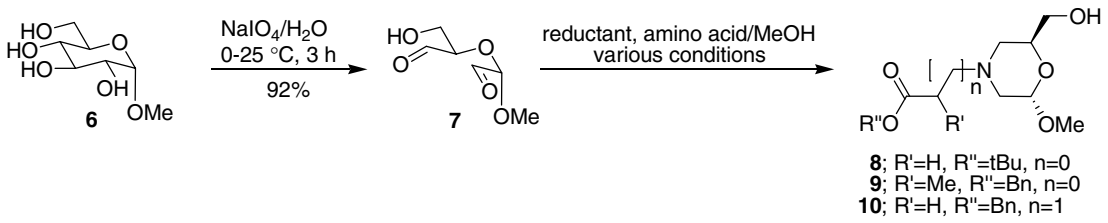
doi:10.1016/j.tetlet.2006.10.133




**Scheme 1.**




**Scheme 2.**

the synthesis of which is outlined in Scheme 1. The main synthetic issues to be addressed were orthogonal protection of the functionalities and the need for a practical, high-yielding synthesis. The main drawback of this synthesis was the $NaCNBH_3$-mediated double reductive amination (Borch reduction) of dialdehyde **3** with a protected amino acid, which in our hands gave irreproducible and moderate yields, even after long reaction times. Comparable moderate yields and long reaction times (4–24 h) with similar reactions have been reported, especially when using (protected) amino acids as the donors of the amino moiety.[3,4,7] Even careful analysis of the reaction mixture did not enable Clark and Osborn to offer any explanation for the low yields of the double reductive amination step.[7] This prompted us to examine systematically a model reaction starting from α-D-methylglucoside **6** (Scheme 2), in an attempt to increase the yields and reduce reaction times.

The initial dialdehyde **7** was obtained by the oxidation of α-D-methylglucoside **6** with excess $NaIO_4$.[†] Clark and Osborn reported insufficient transformation of the desired dialdehyde and further formation of oxazepanes.[7] Therefore, they recommended an increase of $NaIO_4$ to 5 molar equiv. In our hands, 2 molar equiv yielded the desired product, as demonstrated by TLC and by the products formed in the next step.

In the first series of morpholines synthesized (Table 1), double reductive amination reactions were carried out with dialdehyde **7**, protected amino acids and a large excess of the reductant at room temperature.[‡] In order to circumvent the possible formation of open chain diamines, we carried out reactions in diluted reaction mixtures in carefully adjusted acidic media, with molar equivalents of dialdehyde **7** increasing to 3. Furthermore, the protected amino acid and dialdehyde **7** were allowed to react for 30 min before the addition of the reductant, and without molecular sieves or other dehydrating agents—in contrast to some previously reported procedures.[7,8] The presence of water in the reaction mixture allowed equilibrium to be achieved between amine–imine and amine–aldehyde, which resulted in the desired amine–morpholine with no trace of acyclic diamine by-product.

[†] A solution of sodium periodate (2 equiv) in distilled water was added dropwise to an ice-cooled water solution of α-D-methylglucoside **6** (1 equiv). The reaction mixture was stirred for another 4 h at room temperature and concentrated in vacuo. The resulting white solid was washed with tetrahydrofuran and subjected to ultrasound for 5 min (3×). The organic fractions were collected, filtered through a Fluoropore™ (PTFE) membrane filter (0.5 μm pore size) and the solvent evaporated in vacuo. The residual colourless syrup was treated with diethyl ether and the solvent evaporated (3 × 30 mL) to yield a white amorphous solid. The crude product was immediately used in the next step.

[‡] *Typical procedure for double reductive amination under normal conditions:* To a solution of 3-hydroxy-2-(1-methoxy-2-oxoethoxy)propanal (dialdehyde **7**) in methanol (10 mL), the protected amino acid (in the form of a salt) was added and the acidity of the solution adjusted with glacial acetic acid to pH 5 (wet pH-indicator strip). After stirring for 30 min, the reductant (sodium cyanoborohydride or sodium triacetoxyborohydride) was added and stirring was continued overnight (15 h). The mixture was concentrated in vacuo, and the residue dissolved in ethyl acetate (30 mL) and washed with a saturated solution of sodium hydrogen carbonate (3 × 10 mL), water (1 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over sodium sulphate, the solvent evaporated in vacuo and the residue further purified by column chromatography (silica gel, dichloromethane/methanol = 20/1) to yield morpholino derivatives **8**–**10**.

**Table 1.** Morpholino-glycopeptides **8**–**10** obtained according to Scheme 2

| Entry | Reductant | Dialdehyde (molar equiv)[a] | Amino acid | Yield[a,c] (%) |
|---|---|---|---|---|
| **8a** | 4.0 equiv $NaCNBH_3$ | 0.9 | Gly*O*t*Bu* × HCl | 14 |
| **8b** | 4.0 equiv $NaCNBH_3$ | 1.5 | Gly*O*t*Bu* × HCl | 38 |
| **8c** | 5.0 equiv $NaCNBH_3$ | 3.0 | Gly*O*t*Bu* × HCl | 39 |
| **8d** | 4.0 equiv $Na(AcO)_3BH$ | 1.5 | Gly*O*t*Bu* × HCl | 32 |
| **9a** | 4.0 equiv $NaCNBH_3$ | 0.9 | L-AlaOBn × TsOH[b] | 22 |
| **9b** | 4.0 equiv $NaCNBH_3$ | 1.5 | L-AlaOBn × TsOH | 42 |
| **9c** | 5.0 equiv $NaCNBH_3$ | 3.0 | L-AlaOBn × TsOH | 50 |
| **9d** | 10 equiv $NaCNBH_3$ | 3.0 | L-AlaOBn × TsOH | 52 |
| **9e** | 4.0 equiv $Na(AcO)_3BH$ | 0.9 | L-AlaOBn × TsOH | 19 |
| **9f** | 4.0 equiv $Na(AcO)_3BH$ | 1.5 | L-AlaOBn × TsOH | 33 |
| **9g** | 5.0 equiv $Na(AcO)_3BH$ | 3.0 | L-AlaOBn × TsOH | 44 |
| **10a** | 4.0 equiv $NaCNBH_3$ | 0.9 | β-AlaOBn × TsOH | 26 |
| **10b** | 4.0 equiv $NaCNBH_3$ | 1.5 | β-AlaOBn × TsOH | 50 |
| **10c** | 5.0 equiv $NaCNBH_3$ | 3.0 | β-AlaOBn × TsOH | 59 |
| **10d** | 4.0 equiv $Na(AcO)_3BH$ | 1.5 | β-AlaOBn × TsOH | 32 |

[a] Calculated relative to the amino acid.
[b] TsOH = *p*-toluenesulfonic acid.
[c] Refers to the yield of the purified product.

**Table 2.** Morpholino-glycopeptides **8**–**14** obtained as in Scheme 2 with microwave-assisted chemistry

| Entry | Reductant | Dialdehyde[a] (molar equiv) | Amino acid/amine | Yield[a,b] (%) | Time (min) |
|---|---|---|---|---|---|
| **8e** | | | Gly*O*t*Bu* × HCl | 40 | 10 |
| **9h** | | | L-AlaOBn × TsOH | 37 | 5 |
| **9i** | | | L-AlaOBn × TsOH | 41 | 10 |
| **9j** | | | L-AlaOBn × TsOH | 41 | 15 |
| **10e** | 4.0 equiv $NaCNBH_3$ | 1.5 | β-AlaOBn × TsOH | 56 | 10 |
| **11** | | | $EtOOC(CH_2)_3NH_2$ × HCl | 57 | 10 |
| **12** | | | $BnNH_2$ | 64 | 10 |
| **13** | | | $PhNH_2$ | 88 | 10 |
| **14** | | | 4-*n*-$BuC_6H_4NH_2$ | 73 | 10 |

[a] Calculated relative to the amino acid.
[b] Refers to the yield of the purified product.

The reports of procedures involving different starting quantities of the dialdehyde and amino acid encouraged us to explore reaction yields as a function of the quantity of reactants.[3,4,7,8] The results (Table 1) clearly demonstrate that yields increased with increasing molar equivalents of dialdehyde **7**, and yields of up to 59% were achieved in this way (**8c**, **9d**, **10c**)[10], which is a reasonable improvement over the yields of Clark and Osborn (29–33%).[7] When calculating yield relative to both the dialdehyde (which is usually more laborious to obtain) and the amino acid, an optimum reagent ratio of dialdehyde:amino acid = 1.5:1 was obtained. Raising the quantity of $NaCNBH_3$ to 10 molar equiv did not significantly influence the overall yield.

A further reason for the earlier moderate yields could be the competitive reduction of dialdehyde **7** or the intermediate amine–aldehyde, with sodium cyanoborohydride, to give the resulting alcohol. The use of the milder reductant, sodium triacetoxyborohydride, should therefore diminish the extent of this side reaction. This, however, failed to produce any improvement in yield (**8d**,**9e**–**g**,**10d** compared to **8b**,**9a**–**c**,**10b**).

As long reaction times of up to 24 h have been reported for a complete double reductive amination,[7,8,11] we reasoned that conducting the reaction under microwave irradiation should significantly shorten the reaction times. The reactions were shown to be complete after heating the reaction mixtures for only 10 min at 100 °C under microwave irradiation (**9h**–**j**).[§] The products were obtained in yields (Table 2) similar to those for the reactions performed at room temperature (**8b**, **9b**, **10b** compared to **8e**, **9i**, **10e**). Although reactions were conducted in methanol under acidic conditions, no transesterification products were identified and the

[§] *Typical procedure for microwave-assisted double reductive amination:* To a solution of dialdehyde **7** in methanol (3 mL) the amine donor was added and the acidity of the solution adjusted with glacial acetic acid to pH 5 (wet pH-indicator strip). Sodium cyanoborohydride was added and the mixture heated in a dedicated microwave reactor (CEM™Discover) at 100 °C using a power of 20 W for the reported time. The power was allowed to fluctuate as the temperature reached the desired value. Afterwards, the reaction mixture was concentrated in vacuo, and the residue dissolved in ethyl acetate (30 mL) and washed with a saturated solution of sodium hydrogen carbonate (3 × 10 mL), water (1 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over sodium sulphate, the solvent evaporated in vacuo and the residue further purified by column chromatography (silica gel, dichloromethane/methanol = 20/1) to yield morpholino derivatives **8**–**14**.

isolated morpholines were single isomers, as shown by NMR. In order to test the general applicability of our method, other alkyl and aryl amines were used (Table 2, compounds **11**–**14**). Surprisingly, the highest yields were obtained with aromatic amines.

In conclusion, we have reported an improved synthetic route to hybrid morpholino-glycoamino acids through double reductive amination. The microwave-assisted procedure results in a reasonable improvement in the reaction yield and the shortest reaction times reported so far. The optimized procedure offers a feasible route for multi-gram synthesis of orthogonally protected functionalized morpholino-glycoamino acids, the synthesis of which will be reported in due course.

## Acknowledgements

This work was supported by the European Union FP6 Integrated Project EUR-INTAFAR (Project No. LSHM-CT-2004-512138) under the thematic priority Life Sciences, Genomics and Biotechnology for Health, and the Ministry of Education, Science and Sport of the Republic of Slovenia. The author wishes to thank Dr. Roger Pain for the critical reading of the manuscript.

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10. *Representative examples:* Compound **8**: colourless oil. IR (ATR-IR, thin film, $cm^{-1}$): 3432, 2922, 2840, 1723, 1447, 1373, 1217, 1155, 1038, 898, 832, 739. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ (ppm) = 1.47 (s, 9H, *t*-Bu), 1.93 (t, 1H, –OH, $J = 5.4$ Hz), 2.55 (t, 1H, H-$5_{ax}$, $J_{5ax,6} = J_{5ax,5eq} = 10.8$ Hz), 2.68 (dd, 1H, H-$3_{ax}$, $J_{3ax,2} = 2.7$ Hz, $J_{3ax,3eq} = 11.4$ Hz), 2.80 (m, 1H, H-$5_{eq}$), 2.94 (m, 1H, H-$3_{eq}$), 3.17 (d, 1H, NC*H*H′COO*t*Bu, $J_{H,H'} = 17.0$ Hz), 3.23 (d, 1H, NCH*H*′COO*t*Bu, $J_{H,H'} = 17.0$ Hz), 3.43 (s, 3H, $CH_3O$), 3.66 (m, 2H, C$H_2$OH), 4.13 (m, 1H, H-6), 4.74 (br s, 1H, H-2). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ (ppm) = 28.52 (($CH_3$)$_3$C), 53.41 ($CH_3O$), 55.12 (C-5), 55.29 (N$CH_2$COO*t*Bu), 59.67 (C-3), 64.22 (CH$CH_2$OH), 69.25 (C-6), 81.86 ((CH$_3$)$_3$*C*), 97.37 (C-2), 169.94 (CO). MS (EI) *m*/*z*: 261 $(M)^+$. HRMS Calcd for $C_{12}H_{23}NO_5$ *m*/*z*: 261.158030 $(M)^+$. Found 261.157623. Compound **9**: colourless oil. IR (ATR-IR, thin film, $cm^{-1}$): 3420, 2922, 2821, 1735, 1451, 1370, 1256, 1158, 1124, 1046, 984, 886, 805, 727, 688. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ (ppm) = 1.35 (d, 3H, C$H_3$CH, $J = 7.2$ Hz), 2.08 (br s, 1H, –OH), 2.49 (t, 1H, H-$5_{ax}$, $J_{5ax,6} = J_{5ax,5eq} = 10.8$ Hz), 2.65 (dd, 1H, H-$3_{ax}$, $J_{3ax,2} = 2.7$ Hz, $J_{3ax,2} = 11.7$ Hz), 2.75 (m, 1H, H-$5_{eq}$), 2.92 (br d, 1H, H-$3_{eq}$, $J_{3eq,2} = 11.7$ Hz), 3.41 (s, 3H, $CH_3O$), 3.41 (q, 1H, $CH_3CH$, $J = 7.2$ Hz), 3.55 (dd, 1H, CHC*H*H′OH, $J_1 = 3.8$ Hz, $J_2 = 11.7$ Hz), 3.64 (dd, 1H, CHCH*H*′OH, $J_1 = 5.6$ Hz, $J_2 = 11.7$ Hz), 4.06 (m, 1H, H-6), 4.71 (br s, 1H, H-2), 5.12 (s, 2H, PhC$H_2$O), 7.36 (m, 5H, Ph). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ (ppm) = 15.15 ($CH_3$CH), 50.23 (C-5), 52.48 ($CH_3O$), 55.44 (C-3), 62.55 ($CHCH_3$), 64.29 (CH$CH_2$OH), 66.54 ($CH_2$Ph), 69.50 (C-6), 97.60 (C-2), 128.72, 128.97, 136.17 (C–Ar), 172.84 (CO). MS(EI) *m*/*z*: 309 $(M)^+$. HRMS Calcd for $C_{16}H_{23}NO_5$ *m*/*z*: 309.158560 $(M)^+$. Found 309.157623.
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