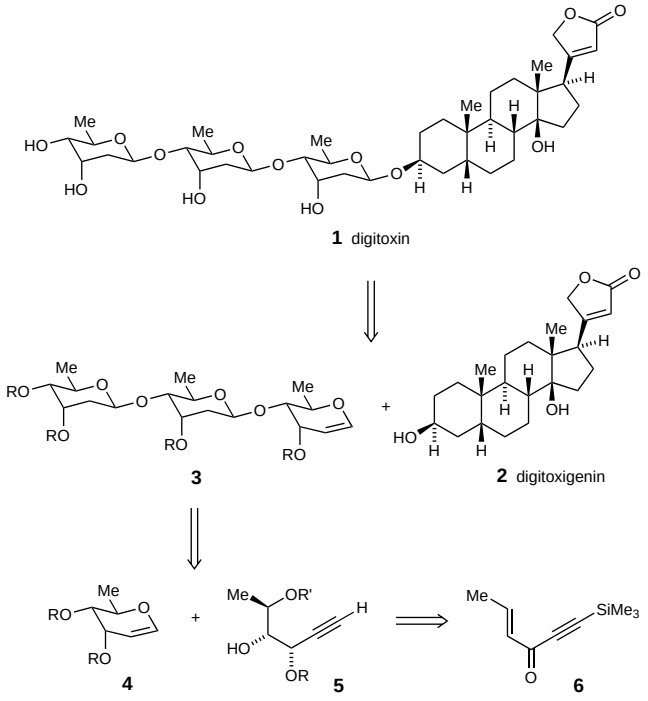
Convergent Synthesis of Digitoxin: Stereoselective Synthesis and Glycosylation of the Digoxin Trisaccharide Glycal**

Frank E. McDonald* and K. Subba Reddy

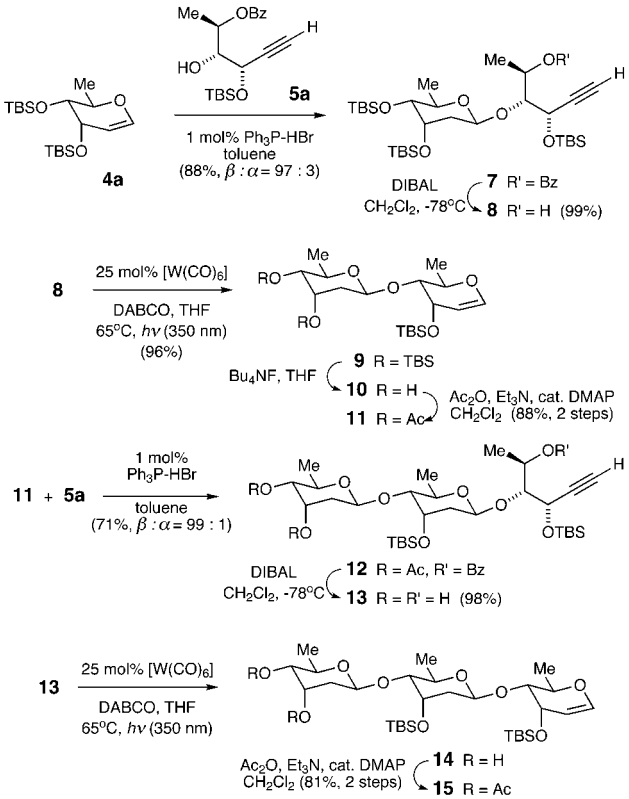
Extracts from the leaves of *Digitalis* (foxglove) plants have long been used to strengthen the force and velocity of heart muscle contraction, and the pure compound digitoxin (**1**, Scheme 1) is widely prescribed for treating congestive heart



Scheme 1. Retrosynthesis of digitoxin (**1**).

failure and cardiac arrhythmia.[1] Unfortunately the therapeutic dose for digitoxin (14–26 ng mL^{-1}) is dangerously near the toxic dose (>35 ng mL^{-1}), thus patients receiving digitalis therapy require careful medical monitoring.[2] Wiesner and co-workers reported the first and only chemical synthesis of digitoxin.[3] This landmark synthesis featured construction of the natural product by sequential glycosylations with D-digitoxose monosaccharide glycosyl donors, and suffered from the requirement that the butenolide was masked as a furan derivative for glycosylation and protective group removal steps. We recognized that a significantly enhanced and convergent synthesis could be accomplished by direct attachment of a preformed trisaccharide to the butenolide-containing digitoxigenin aglycone **2**. In preparation for a study of the structure–activity relationships for the oligosaccharide substructure of digitalis glycosides, we now report the synthesis of trisaccharide glycal **3** by iterative alkynol cycloisomerization[4] and acid-catalyzed glycosylation to form 2-deoxyglycosides. We also describe herein the first direct coupling of a digitoxose trisaccharide to digitoxigenin aglycone **2**.

Our synthesis begins with protic acid-catalyzed stereoselective glycosylation of monosaccharide 6-deoxy-D-*ribo* glycal **4a** with alkynyl alcohol **5a** (Scheme 2).[5] Our initial



Scheme 2. Stereoselective synthesis of digitoxose trisaccharide glycal **15**. TBS = *tert*-butyldimethylsilyl, DIBAL = diisobutylaluminum hydride, DABCO = 1,4-diazabicyclo[2.2.2]octane, DMAP = 4-dimethylaminopyridine.

studies demonstrated that camphorsulfonic acid (CSA) is a suitable catalyst for this transformation, but more extensive studies indicate that Ph_3P–HBr is an even better catalyst with regard to reaction rate (6 h vs. 12–18 h with CSA) and reproducibility at multimillimole scales, as well as providing the 2-deoxyglycoside **7** with excellent anomeric stereoselectivity (Scheme 2). Reductive debenzoylation and tungsten carbonyl catalyzed *endo*-selective cycloisomerization of alkynol substrate **8** gave the disaccharide glycal **9**.

Acid-catalyzed glycosylation of this disaccharide **9** with alkynol alcohol **5a** proceeded with low yield and poor anomeric selectivity (β:α = 76:24, ca. 30% combined yield) relative to glycosylation of the monosaccharide glycal **4a**

[*] Prof. Dr. F. E. McDonald, Dr. K. S. Reddy
Department of Chemistry
Emory University
1515 Pierce Drive
Atlanta, GA 30322 (USA)
Fax: (+1) 404-727-6586
E-mail: fmcdona@emory.edu

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(β:α = 97:3, 88% yield of the β-anomer). However, we observed excellent stereocontrol and good yield in the corresponding glycosylation of the disaccharide **11** bearing acetate protective groups at the non-reducing sugar to afford disaccharide glycoside **12** (β:$\alpha \geq$ 99:1, 71% yield; Scheme 2). These results demonstrate the sensitivity of acid-catalyzed glycosylations to remote substituent effects.[6] To the best of our knowledge, this represents the first report of acid-catalyzed glycosylations on disaccharide glycals, as all previous reports have been limited to monosaccharide glycal substrates.[7] In the course of these glycosylations, we observed that better β-selectivity is obtained when toluene is the solvent rather than CH_2Cl_2 or other solvents.

As selective removal of the benzoate at O5 of the alkynyl glycoside would have been difficult, we simply removed all ester protective groups from **12** and explored tungsten carbonyl catalyzed cycloisomerization of the alkynyl *triol* substrate **13**. This transformation gave trisaccharide glycal **14** without interference from the more distant hydroxy groups. Product **14** was purified after capping the two remaining hydroxy groups as acetate esters to give **15**.

Acid-catalyzed glycosylation of the trisaccharide glycal **15** with digitoxigenin aglycone **2**[8] was complicated by insolubility of **2** in toluene. Ph_3P–HBr-catalyzed glycosylation in $CHCl_3$ provided a 60:40 mixture of 2′-deoxyglycoconjugate **16**β along with the α-anomer (Scheme 3; Table 1, entry 2), and the anomers could be separated by silica gel chromatography. Slightly better anomeric selectivity (β:α = 73:27) was observed with 1,2-dichlorobenzene as solvent (Table 1, entry 4), although in this case the yields of **16** were diminished by poor solubility of the aglycone **2**. Deprotection of the TBS protective groups of **16**β initially proved challenging, as the cycloisomerization and glycosylation steps placed the O3 silyl ethers of the two central digitoxose sugars in sterically hindered positions.[9] However, ammonium hydrogen fluoride in dimethylformamide (DMF)/*N*-methylpyrrolidine (NMP) solvent[10] proved mild enough to remove both TBS ethers from **16**β in the presence of the fluoride-sensitive butenolide and acid-sensitive 2-deoxyglycoside linkages. The remaining acetate protective groups of **17**β were then removed by basic methanolysis[11] to provide digitoxin **1** which was identical in all respects to a commercially available sample (Aldrich Chemical Company, TLC, melting point, ^{1}H and ^{13}C NMR, and optical rotation).

In conclusion, we have accomplished a highly convergent and stereoselective synthesis of digitoxin **1**. Notable features include iterative application of a tungsten-catalyzed *endo*-selective alkynol cyclomerization methodology, and the first recorded examples of acid-catalyzed glycosylations of disaccharide and trisaccharide glycals. Studies in progress include the synthesis and biological evaluation of additional oligosaccharide stereoisomers of digitoxin.

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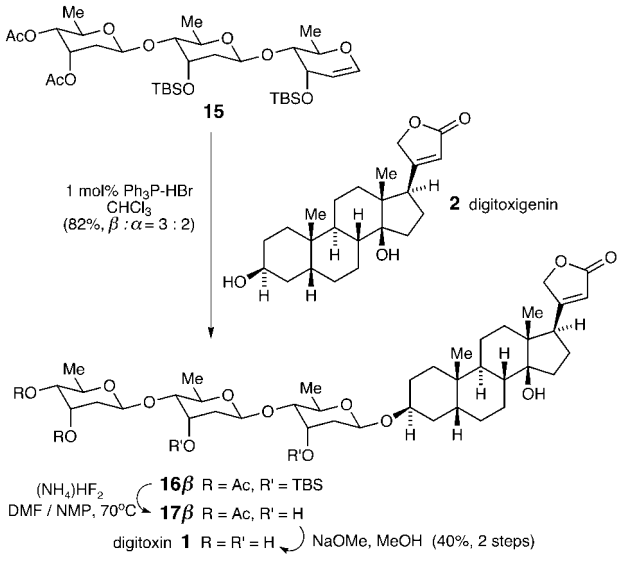

Scheme 3. Glycosylation of **15** and synthesis of digitoxin **1**. DMF = *N*,*N*′-dimethylformamide, NMP = *N*-methylpyrrolidine.

Table 1. Glycosylation of trisaccharide glycal **15** with (+)-digitoxigenin **2**.

Entry	Catalyst[a]	Solvent	**16**β:**16**α	Combined yield [%] **16**β + **16**α
1	CSA	CH_2Cl_2	60:40	68
2	Ph_3P–HBr	$CHCl_3$	60:40	82
3	Ph_3P–HBr	toluene:$CHCl_3$ (2:1)	64:36	71
4	Ph_3P–HBr	1,2-dichlorobenzene	73:27	54

[a] In each case 1 mol% of catalyst was used.

[1] "Cardiac Glycosides, Part 1: Experimental Pharmacology": in *Handbook of Experimental Pharmacology*, *Vol. 56*, Springer, Berlin, **1981**.

[2] *Drug Facts and Comparisons*, 50th ed., Facts and Comparisons, St. Louis, MO, **1996**, pp. 675–684.

[3] a) K. Wiesner, T. Y. R. Tsai, H. Jin, *Helv. Chim. Acta* **1985**, *68*, 300; b) K. Wiesner, T. Y. R. Tsai, *Pure Appl. Chem.* **1986**, *58*, 799.

[4] F. E. McDonald, K. S. Reddy, Y. Díaz, *J. Am. Chem. Soc.* **2000**, *122*, 4304.

[5] Compounds **4a** and **5a** were obtained enantiomerically pure (>99% *ee*) from enynone **6**, in seven and five steps, respectively.[4]

[6] Kunz and Unverzagt have demonstrated that acetate protective groups provide significant stabilization of acid-sensitive glycosides. This effect may operate by inductively decreasing basicity of the glycosidic oxygen, although Kunz and Unverzagt have also proposed that protonation of the acetate carbonyl oxygen atoms results in Coulombic repulsion, which hinders protonation of the glycoside and ring oxygen atoms and thus "protects" the glycoside bond from acid-catalyzed hydrolysis. a) H. Kunz, C. Unverzagt, *Angew. Chem.* **1988**, *100*, 1763; *Angew. Chem. Int. Ed.* **1988**, *27*, 1697; b) C. Unverzagt, H. Kunz, *Bioorg. Med. Chem.* **1994**, *2*, 1189; c) F. E. McDonald, S. J. Danishefsky, *J. Org. Chem.* **1992**, *57*, 7001.

[7] The reaction of alcohols with 3,4-dihydro-2*H*-pyran has long been known for the introduction of tetrahydropyran (THP) protective groups, but only more recently has this reaction been employed in reactions of alcohols with more complex dihydropyrans (carbohydrate glycals). a) Catalyzed by camphorsulfonic acid: K. Toshima, K. Tatsuta, M. Kinoshita, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2369; PPh_3-HBr: b) V. Bolitt, C. Mioskowski, S.-G. Lee, J. R. Falck, *J. Org. Chem.* **1990**, *55*, 5812; c) N. Kaila, M. Blumenstein, H. Bielawska, R. W. Franck, *J. Org. Chem.* **1992**, *57*, 4576; d) C. J. France, I. M. McFarlane, C. G. Newton, P. Pitchen, M. Webster, *Tetrahedron Lett.* **1993**, *34*, 1635; e) acid resin: S. Sabesan, S. Neira, *J. Org. Chem.* **1991**, *56*, 5468.

[8] Digitoxigenin (**2**) used in this study was purchased from Aldrich Chemical Company. a) Total synthesis of **2**: G. Stork, F. West, H. Y. Lee, R. C. A. Issacs, S. Manabe, *J. Am. Chem. Soc.* **1996**, *118*, 10660;

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b) a representative partial synthesis of **2**: S. F. Donovan, M. A. Avery, J. E. McMurry, *Tetrahedron Lett.* **1979**, *20*, 3287.
[9] Tetra-*n*-butylammonium fluoride/THF resulted in destruction of the butenolide, whereas HOAc/aq. THF gave no reaction at room temperature. At higher temperatures (60 °C) desilylation was accompanied by partial hydrolysis of the acid-sensitive 2-deoxyglycoside linkages.
[10] M. Seki, K. Kondo, T. Kuroda, T. Yamanaka, T. Iwasaki, *Synlett* **1995**, 609.
[11] M. Luta, A. Hensel, W. Kreis, *Steroids* **1998**, *63*, 44.

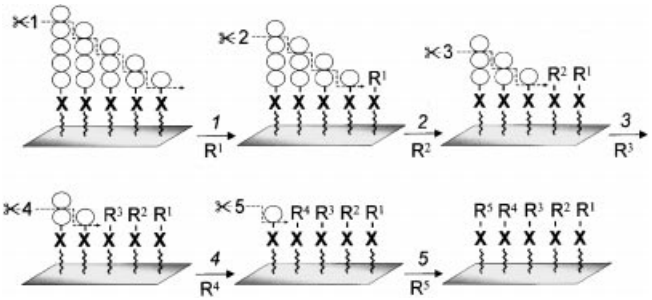

Figure 1. UCP exemplified by the deprotection and selective derivatization of five functional groups (R^{1-5}). The functional groups (**X**) are protected with a concatenated set of oligomeric protecting groups (○). The sequential removal of each protecting unit is achieved with a single reaction type. Selective derivatization of each newly unmasked functional group is performed after each deprotection step. This iterative process is repeated until all functional groups are deprotected and derivatized as desired.

Unichemo Protection: A Concept for Chemical Synthesis**

Les P. Miranda and Morten Meldal*

Chemical synthesis is a powerful method for creating complex molecules with tailored biological and physical properties for drug discovery, engineering, nanotechnology, and the investigation of biological processes. However, the applicability of chemical synthesis to peptides, oligosaccharides, and other organic molecules is limited and inherently complicated using the existing functional-group protecting strategies. The differential protection of functional groups of similar reactivity in chemical synthesis is a major challenge with conventional protecting-group strategies, namely orthogonal protection and modulated lability.[1–3] In particular, the development of effective protective schemes for polyfunctional molecules is not trivial.[4] The number and type of protecting groups influences the length, efficiency, and complexity of a given synthesis, and is often responsible for its success or failure.

Herein, a new concept, termed unichemo protection (UCP), illustrated in Figure 1, is introduced. This strategy only requires a single chemical process for all deprotection reactions. The UCP protecting groups are derived from a repetitive unit that permits their controlled and efficient stepwise removal. Functional site selectivity is achieved by varying the degree of oligomerization at each site, and, after each deprotection cycle, only the newly liberated functional site is available for derivatization.

In principle, the UCP strategy does not impose a restriction on the possible number of selectively protected sites in a molecule. This method should be particularly useful in areas including the combinatorial synthesis of highly substituted scaffolds, peptide synthesis, template-assisted synthetic proteins (TASP),[5, 6] automated oligosaccharide synthesis,[7] and the general goal of automated organic synthesis.

The effectiveness of the UCP chemistry was demonstrated by the controlled derivatization of a pentalysine-based amino-functionalized scaffold on the solid support. To facilitate this, a *N-sec*-butylglycyl-based protecting-group unit was devised for the protection of amino groups.[8] With conventional protection strategies, the controlled derivatization of five or more otherwise identical amino groups on the solid-support is a difficult challenge. Here, this problem was solved by using the UCP concept in the form of N^{ε}-oligo(*N-sec*-butylglycyl) protected lysine building blocks[9] for the assembly of scaffold **1** (see Figure 3).[10]

In its present form the UCP concept takes advantage of large reactivity differences between primary amino functional groups and the otherwise similar protecting groups. The use of oligomeric *N-sec*-butylglycyl protecting groups exploits the relatively high degree of steric hindrance around the secondary amino terminus to differentiate between deprotection and derivatization processes. High yields of oligomeric *N-sec*-butylglycyl protecting groups are readily obtained using strong activation during amide-bond formation on the solid support.[11] Importantly, the oligomers were completely inert to less activated carboxylic derivatives, such as readily prepared *para*-nitrophenyl (ONp) and succinimide (OSu) esters (Figure 2).[12, 13] The inert character of the UCP secondary amine protecting groups under acylation conditions thus allows for the chemoselective derivatization of newly liberated primary amino group with nitrophenyl esters. That is, chemical selectivity against the secondary amino terminus of the protecting groups is employed to distinguish between derivatization and removal steps.

For deprotection cycles, efficient stepwise removal of terminal protecting group units is facilitated by a reliable two-step procedure originally developed by Edman for protein sequencing.[14] In the first step, phenylisothiocyanate (PITC) reacts quantitatively at pH 8 with the terminal unit of the oligomeric protecting group (Figure 2). In the second step, a quantitative cyclization and elimination reaction occurs at

[*] Prof. M. Meldal, Dr. L. P. Miranda
Department of Chemistry
Carlsberg Laboratory
Gamle Carlsberg Vej 10, Copenhagen (Denmark)
Fax: (+45) 3327-4708
E-mail: mpm@crc.dk

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