An Access to Glycoconjugate Libraries through Multicomponent Reactions

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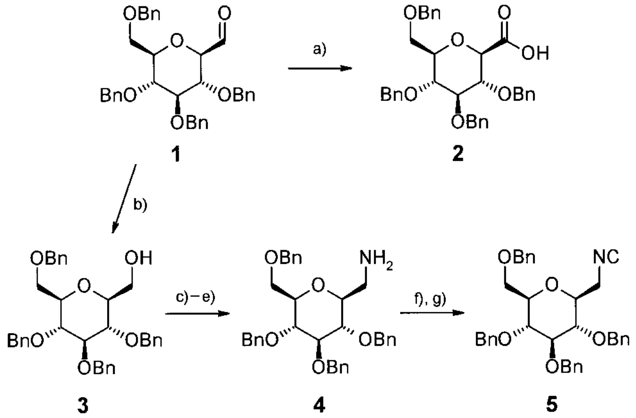
A new approach to the identification of lead structures for drug development is the combinatorial synthesis of compound libraries and their investigation in high-throughput screening (HTS) systems. In this way libraries of peptides,[1] oligonucleotides,[2] and other low molecular weight compounds[3] were built-up and studied. In contrast, and despite their biological importance,[4] libraries of oligosaccharides could not be investigated in HTS systems. Today's methods of glycoside and oligosaccharide syntheses[5] are compatible with demands for automatic syntheses only to a very limited extend. However, several new approaches presented recently[6] are very encouraging and will bring new solutions in the near future.

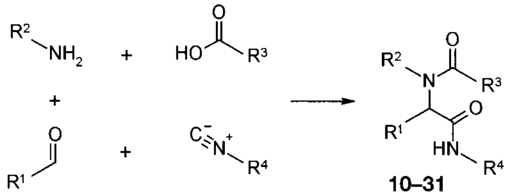
For drug design it is of particular interest that only substructures of oligosaccharides are involved in most carbohydrate–protein interactions. Several studies have shown that individual arrays of functional groups possibly located on different monosaccharide moieties are recognized by complementary receptors.[7] This is the rational basis for the design of glycomimetics which carry their essential functional groups in a matching spatial arrangement.[8] This further implies that the individual carbohydrate moieties do not have to be linked through glycosidic linkages which are difficult to couple. Instead, they can be linked through other bond types as long as the presentation of the essential functional groups is not restricted.

A suitable method for the generation of libraries of low molecular weight compounds in solution is the application of multicomponent condensations (MCC).[9] Combinatorial reactions such as the Ugi reaction[10] should also be suitable for producing glycoconjugate libraries if appropriate carbohydrate derivatives having aldehyde, amino, carboxylic acid, and isocyanide groups are used. This should open an effective access to libraries of glycomimetics and glycoconjugates. Here we report on our efforts to develop a MCC-based combinatorial synthesis of glycoconjugate libraries.[11] Meanwhile, solid-phase Ugi reactions with C-glycosyl aldehydes have been presented.[12]

In exploratory studies per-O-benzylated carbohydrate building blocks with *β*-D-*gluco* configuration were chosen (Scheme 1). The starting compound was glycosyl aldehyde **1**, which was obtained in good yields from tetra-*O*-benzyl-D-gluconolactone by addition of dithiane.[13] The oxidation of **1** under Masamune conditions[14] yielded the glycosylcarboxylic acid **2**. The reductive amination of **1** did not afford the glycosylmethylamine **4**[15] selectively, but rather led to **4** and the alcohol **3**. Therefore **1** was reduced to **3**, which was transformed into **4** by classical tosylation, azide substitution, and selective reduction. N-Formylation of **4** and dehydration afforded the isocyanide **5**. The C-glycosides **1**, **2**, **4**, and **5** represent the desired carbohydrate prototypes for the Ugi condensation. It was now planned to investigate their synthetic suitability in a model reaction (Scheme 2). One such reaction is that of benzaldehyde (**6**), *p*-methoxybenzylamine (**7**), acetic acid (**8**), and cyclohexylisocyanide (**9**) leading to **10**[16] by stepwise substitution of one or more non-carbohydrate components (Table 1). The condensation of the glycosyl aldehyde **1** with **7**–**9** afforded the diastereomers **11h** and **11l**,[17] which could be separated by column chromatography. Condensation of the glycosylmethylamine **4** led to diasteromers **12**, which could not be separated. The carboxylic acid **2** gave the amides **13h** and **13l**. The yields of these reaction were between 70 and 85%. Both diasteromers were formed in equal amounts. Glycosylisocyanide **5**, however, did not show conversion into condensation product **14** under these conditions even after several days. Amide **14** was produced only in moderate yields after addition of zinc chloride. Apparently the primary isocyanide **5** is only of low nucleophilicity. Nevertheless it should be emphasized that all components reacted as expected and should therefore be suitable for an assembly of glycoconjugate libraries.



Scheme 1. Synthesis of C-glycoside building blocks **1**, **2**, **4**, and **5** for the four-component reactions. a) $KMnO_4$, *t*BuOH, H_2O, NaH_2PO_4, pH 4.5, 81%; b) $LiBH_4$, THF, 95%; c) TsCl, pyridine; d) NaN_3, DMF; e) $NaBH_4$, $NiCl_2$, EtOH, dioxane, 71% (over three steps); f) HCO_2Me, 40°C, 87%; g) $(Cl_3CO)_2CO$, DABCO, CH_2Cl_2, 60%. Bn = benzyl, DABCO = 1,4-diazabicyclo[2.2.2]octane, Ts = toluenesulfonyl.



Scheme 2. The Ugi reactions by which four components react to provide a diamide; for R^1–R^4, see Table 1.

We next addressed the combination of two or more carbohydrate building blocks in Ugi reactions to afford higher glycosylated amino acid derivatives. The synthesis of the six possible diglycosylated compounds **15**–**20** was studied first. Glycosyl aldehyde **1** and amine **4** together with **8** and **9** gave

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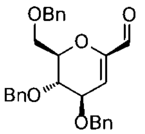
1433-7851/98/3724-3436 $ 17.50+.50/0

Table 1. Condensation products of the Ugi reactions with carbohydrate building blocks. A = 2,3,4,6-tetra-*O*-benzyl-*β*-D-glucopyranosyl, B = 2,3,4,6-tetra-*O*-benzyl-*β*-D-glucopyranosyloxymethyl, PMB = 4-methoxybenzyl, **h**,**l** = diastereomers separated by chromatography into the higher (**h**) and lower (**l**) running stereoisomers, n.d. = not determined.

Aldehyde	Amine	Acid	Isocyanide	Product	R^1	R^2	R^3	R^4	Molecular formula	M_r	MS [*m*/*z*]	^{13}C NMR [δ] NCH,R^1CO	yield [%]
6	**7**	**8**	**9**	**10**	C_6H_5	PMB	CH_3	C_6H_{11}	$C_{24}H_{30}N_2O_3$	394.52	CI: 395 [*M* + H]	63.0	74
1	**7**	**8**	**9**	**11h,l**	A	PMB	CH_3	C_6H_{11}	$C_{52}H_{60}N_2O_8$	841.07	CI: 841 [*M* + H]	62.0, 62.1	41 + 37
6	**4**	**8**	**9**	**12**	C_6H_5	A–CH_2	CH_3	C_6H_{11}	$C_{51}H_{58}N_2O_7$	811.04	CI: 811 [*M* + H]	64.4, 64.2	74
6	**7**	**2**	**9**	**13h,l**	C_6H_5	PMB	A	C_6H_{11}	$C_{57}H_{62}N_2O_8$	903.14	CI: 903 [*M* + H]	63.6, 63.9	36 + 33
6	**7**	**8**	**5**	**14**	C_6H_5	PMB	CH_3	A–CH_2	$C_{53}H_{56}N_2O_8$	849.05	CI: 849 [*M* + H]	63.2	23
1	**4**	**8**	**9**	**15h,l**	A	A–CH_2	CH_3	C_6H_{11}	$C_{79}H_{88}N_2O_{12}$	1257.59	CI: 1257 [*M* + H]	62.3, 62.3	33 + 30
1	**7**	**2**	**9**	**16h,l**	A	PMB	A	C_6H_{11}	$C_{85}H_{92}N_2O_{13}$	1349.69	CI: 1349 [*M* + H]	62.2, 62.4	46 + 41
1	**7**	**8**	**5**	**17**	A	PMB	CH_3	A–CH_2	$C_{81}H_{86}N_2O_{13}$	1295.59	FAB: 1317 [*M* + Na]	n.d.	8
6	**4**	**2**	**9**	**18**	C_6H_5	A–CH_2	A	C_6H_{11}	$C_{84}H_{90}N_2O_{12}$	1319.66	CI: 1319 [*M* + H]	63.8, 63.1	72
6	**4**	**8**	**5**	**19**	C_6H_5	A–CH_2	CH_3	A–CH_2	$C_{80}H_{84}N_2O_{12}$	1265.57	FAB: 1287 [*M* + Na]	n.d.	11
6	**7**	**2**	**5**	**20**	C_6H_5	PMB	A	A–CH_2	$C_{86}H_{88}N_2O_{13}$	1357.67	FAB: 1379 [*M* + Na]	n.d.	18
1	**4**	**2**	**9**	**21**	A	A–CH_2	A	C_6H_{11}	$C_{112}H_{120}N_2O_{17}$	1766.21	FAB: 1787 [*M* + Na]	n.d.	61
1	**4**	**8**	**5**	**22**	A	A–CH_2	CH_3	A–CH_2	$C_{108}H_{114}N_2O_{17}$	1712.12	ESI: 1735 [*M* + Na]	n.d.	Spur
1	**7**	**2**	**5**	**23**	A	PMB	A	A–CH_2	$C_{114}H_{118}N_2O_{18}$	1804.21	ESI: 1827 [*M* + Na]	n.d.	Spur
6	**4**	**2**	**5**	**24**	C_6H_5	A–CH_2	A	A–CH_2	$C_{113}H_{116}N_2O_{17}$	1774.19	ESI: 1797 [*M* + Na]	n.d.	3
1	**4**	**2**	**5**	**25**	A	A–CH_2	A	A–CH_2	$C_{141}H_{146}N_2O_{22}$	2220.74	n.d.	n.d.	0
34	**4**	**2**	**5**	**26**	B	A–CH_2	A	A–CH_2	$C_{142}H_{148}N_2O_{23}$	2250.76	ESI: 2274 [*M* + Na]	n.d.	61
34	**7**	**8**	**9**	**27**	B	PMB	CH_3	C_6H_{11}	$C_{53}H_{62}N_2O_9$	871.09	CI: 871 [*M* + H]	59.3; 57.8	75
6	**37**	**8**	**9**	**28**	C_6H_5	B–CH_2	CH_3	C_6H_{11}	$C_{52}H_{60}N_2O_8$	841.07	CI: 841 [*M* + H]	62.3, 61.7	71
6	**7**	**36**	**9**	**29**	C_6H_5	PMB	B	C_6H_{11}	$C_{58}H_{64}N_2O_9$	933.16	CI: 933 [*M* + H]	63.9; 63.7	74
6	**7**	**8**	**39**	**30**	C_6H_5	PMB	CH_3	B–CH_2	$C_{54}H_{58}N_2O_9$	879.07	CI: 879 [*M* + H]	62.6, 62.1	78
34	**37**	**36**	**39**	**31**	B	B–CH_2	B	B–CH_2	$C_{145}H_{154}N_2O_{26}$	2340.84	MALDI: 2361 [*M* + Na]	n.d.	72

the desired diasteromers **15h** and **15l**. The unsaturated aldehyde **32** was identified as a by-product formed from **1** by *α*,*β*-elimination. The reaction of **1** and glycosylcarboxylic acid **2** together with **7** and **9** afforded **16h** and **16l** after chromatography. Diastereomers **18** obtained in comparable yields from glycosyl building blocks **4** and **2** could not be separated.



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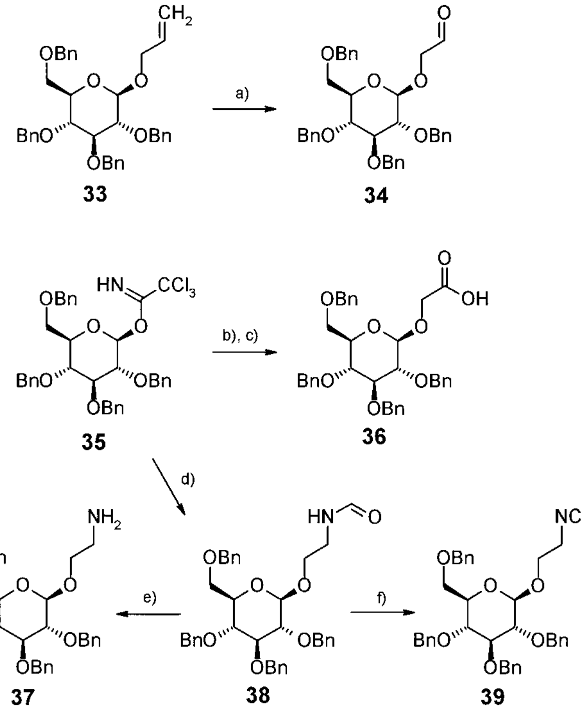
The three following reactions of the isocyanide **5** again reflected its low nucleophility. Combination of **1** and **5** after addition of zinc chloride afforded only 8 % of the desired condensation product **17**; the main product was **32**. Diastereomers **19** were formed in a disappointing 11 % total yield, **20** in only 18 % yield.

The synthesis of the triply glycosylated amino acid derivative **21** from **1**, **4**, and **2** as well as **9** took its expected course in satisfactory yield. The next reactions, however, in which **1** and **5** were combined provided the target products **22** or **23**, respectively, in minute amounts detectable only by mass spectrometry. The main product was **32**. The fourth triply glycosylated amino acid derivative **24** was isolated in very low yields. As expected the synthesis of the fourfold-glycosylated amino acid **25** did not occur. The only isolated product resulting from the combination of the four glycosyl building blocks **1**, **2**, **4**, and **5** was the well-known aldehyde **32**. These results confirm previous findings that a wide application of the Ugi reaction is favored by a high nucleophilicity of the isocyanide component.[10]

Side reactions of the aldehyde component leading to elimination products were expected to be prevented if aldehydes were employed which cannot eliminate. Therefore, glycosyl aldehyde **34** was used, which was obtained from allyl glycoside **33**[18] by ozonolysis (Scheme 3). Indeed, **34** reacted with the glycosyl units **2**, **4**, and **5** in the presence of zinc chloride to provide the fourfold-glycosylated amino acid derivative **26** in more than 60 % yield.

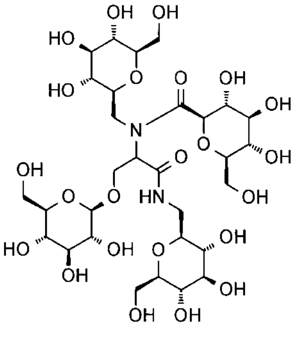


Scheme 3. Synthesis of O-glycosidic building blocks **34**, **36**, **37**, and **39** for the four-component reactions. a) O_3, CH_2Cl_2, Me_2S, −20 °C, 77 %; b) $HOCH_2CO_2Et$, $BF_3 \cdot Et_2O$, THF, 82 %; c) NaOH, MeOH, THF, 72 %; d) $HOCH_2CH_2NHCHO$, $BF_3 \cdot Et_2O$, THF, 68 %; e) NaOH, MeOH, THF, 66 %; f) $(Cl_3CO)_2CO$, DABCO, CH_2Cl_2, 52 %.

A second set of condensation reactions was designed using **34** as well as other O-glycosidic units in order to expand the diversity of the glycoconjugate libraries. The carboxylic acid **36** was obtained by O-glycosylation of ethyl hydroxyacetate with **35**[19] followed by saponification. The corresponding glycosylation of *N*-(2-hydroxyethyl)formamide with **35** afforded **38**. Saponification of **38** provided the amine **37**. Treatment of **38** with triphosgene gave the crystalline isocyanide **39**.

All four model Ugi reactions of these glycosylated building blocks with nonglycoslated partners provided the monoglycosylated amino acid derivatives **27**–**30** in good yields. Side reactions were not observed. Individual diasteromers could be detected with thin-layer chromatography or HPLC, but could not be resolved by preparative column chromatography. They were isolated as 1:1 mixtures. Finally, the condensation of **34**, **36**, **37**, and **39** furnished the desired fourfold-glycosylated amino acid derivate **31**.

The above-mentioned aldehyde, acid, and isocyanide building blocks also reacted smoothly in related Passerini reactions to give singly to triply glycosylated hydroxyacetic acid derivatives. According to further preliminary investigations, analogous reactions using unprotected building blocks provided the condensation products only in significantly lower amounts due to competitive reactions leading to acetals or aminals. O-Acetylated components gave rise to partial N-acetylation of the amine components. The hydrogenolysis of various perbenzylated condensation products in the presence of palladium on charcoal proceeded smoothly and afforded the completely deblocked glycosylated amino or hydroxy acid derivatives, for example **40** and **41**.[20] All products gave mass spectra in accordance with their molecular formula.



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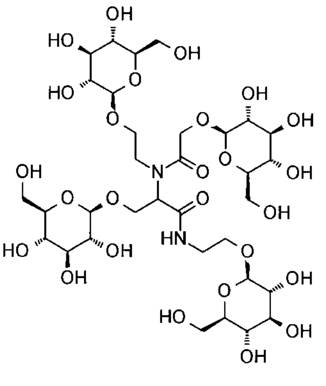

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These reactions have opened a broad access to libraries of diverse glycoconjugate libraries. The combination of various carbohydrate components (with different configurations; C-, O-, or N-glycosidic bonds; different linkers between sugar moiety and functional groups; non-anomeric coupling of the functional groups) carrying aldehyde, acid, amino, and isocyanide groups should offer a huge variety of different branched glycoconjugates as tri- or tetrasaccharide mimetics. But also the mono- or diglycosyl compounds carrying further non-carbohydrate residues, reflecting the vast diversity of organic substituents, are highly important for HTS systems. As these prototype reactions have disclosed, various carbohydrate units can be assembled under similar conditions, thereby enabling automatization of the reactions which have been realized manually until now. It can be predicted that the biological investigation of these saccharide libraries will be of considerable interest.

Experimental Section

Aldehyde (0.05 mmol), amine (0.05 mmol), carboxylic acid (0.05 mmol), and isocyanide (0.05 mmol) were disolved in ethanol (1 mL) and tetrahydrofuran (1 mL) and stirred overnight. The mixture was treated with 1N hydrochloric acid (0.1 mL), stirred for 30 min, and evaporated. The residue was taken up in dichloromethane (5 mL), washed with water (2 mL), dried, evaporated, and purified by column chromatography over silica gel (hexane/ethyl acetate, 7/1 →3/1).

Received: June 25, 1998 [Z12050IE]
German version: *Angew. Chem.* **1998**, *110*, 3634–3637

Keywords: carbohydrates · combinatorial chemistry · glycoconjugates · glycomimetics · multicomponent reactions

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[16] The C atoms from components **6**–**9** gave rise to characteristic ^{13}C NMR signals of condensation products that partially do not exist for glycosylated products. **10**: ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta =$ 172.5 (N*C*OMe), 168.7 (*C*ONHC_6H_{11}), 158.6 (C4 of PMB), 135.4 (C1 of Ph), 130.4–127.4 (arom. *C*H), 113.8 (C3, C5 of PMB), 63.0 (NCH,PhCO), 55.2 (OCH_3), 50.5 (CH_2 of PMB), 48.5 (C1 of C_6H_{11}), 32.8 (C2, C6 of C_6H_{11}), 25.5 (C4 of C_6H_{11}), 24.7 (C3, C5 of C_6H_{11}), 22.5 (CO*C*H_3). The ^{13}C NMR signals of glycosyl residues and O-benzyl groups in glycosylated condensation products were detected between $\delta = 87.5$ and 68.0.

[17] The letters **h** and **l** indicate the higher or lower moving diastereomers in thin-layer chromatography. The absolute stereochemistry was not assigned.

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[20] **40**: ESI-HR-MS: m/z calcd for $C_{30}H_{52}N_2O_{23}Na$: 831.2859 $[M+Na]^+$, found: 831.2889. **41**: ESI-HR-MS: m/z calcd for $C_{33}H_{58}N_2O_{26}Na$: 921.3176 $[M+Na]^+$, found: 921.3199.

1433-7851/98/3724-3439 $ 17.50+.50/0