

Wang Resin-Type Linker Containing a Nitro Group for Polymer Support Oligosaccharide Synthesis: Polymer-Supported Glycosyl Donor

Shino MANABE and Yukishige ITO*

RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama, 351-0198 Japan.

Received May 14, 2001; accepted June 28, 2001

A nitro-introduced Wang resin-type linker for soluble and insoluble polymer support oligosaccharide synthesis is described. The linker was used for connecting glycosyl donors and polymer supports, and was completely stable under the glycosylation conditions tested. The cleavage of the linker was performed under reductive conditions without affecting the protecting groups to release disaccharides.

Key words solid-phase synthesis; oligosaccharide; linker

Solid-phase synthesis is now widely recognized as the leading-edge technology for rapid and efficient oligosaccharide construction.¹⁾ The design of linker that connects the first sugar unit to the polymeric support is of crucial importance for the ultimate success of the synthesis.^{2,3)} We have recently reported a nitro group-introduced Wang resin-type linker, which was useful for connecting soluble polymer support and the glycosyl acceptor.²⁾ An electron-withdrawing nitro group was introduced to attenuate the acid susceptibility of the *p*-alkoxybenzyl ether linkage resin and this linker was proved to be completely stable under typical glycosylation conditions using trimethylsilyl trifluoromethanesulfonate or Cp₂HfCl₂-AgOTf. The products were released with reduction of the nitro group *via* the intramolecular cyclization reaction as hydroxamic acid derivatives. Complete removal of the cyclic hydroxamic acid from the linker was carried out with acid treatment. In this paper, we describe the renewed use of the nitro-modified linker. It connects glycosyl donors and polymer supports, and was designed to be “traceless.” That is, cleavage under reductive conditions directly affords a product with a free hydroxy group at the position originally connected to the linker (Chart 1).

The linker **1**²⁾ was first immobilized *via* ether linkage to poly(ethylene glycol) methyl ether (PEG, average MW 5000) using the imidate method (Chart 2). After hydrolysis of the methyl ester, glycosyl donors **3**–**5** were attached with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (WSCDI) and DMAP to afford **6**–**8** in high yields (Table 1).

The soluble PEG-supported thioglycoside **6** was activated by dimethyl(methylthio)sulfonium triflate (DMTST)⁴⁾ in the presence of 4 eq of acceptor **9**. After reduction of the nitro group by Sn(SPh)₂-PhSH-Et₃N,⁵⁾ product **11** was obtained in 87% overall yield. This structure corresponds to *O*-linked *N*-acetyl-D-glucosamine, which has been found in a variety of nuclear and cytoplasmic proteins.⁶⁾ Reaction with the toluoyl group-protected glucose **10** afforded disaccharide **12** in 86% overall yield after cleavage from the polymer (Table 2). Glycosyl fluoride **7** was reacted with benzyl protected-acceptor **13** by Cp₂HfCl₂-AgOTf⁷⁾ to give **14** in high yield (Chart 3). A combination of *N*-iodosuccinimide-triethylsilyl trifluo-

romethanesulfonate was also compatible with this type of linker, and disaccharide **15** was obtained in 59% yield from glucose-derived donor **8** (Chart 4).

Next, the suitability of the linker for solid-phase oligosaccharide synthesis was examined. To minimize the steric hindrance imposed by solid support, spacer **16** was incorporated between Merrifield resin and the linker (Chart 5). The degree of loading was quantified by elemental analysis. For example, the immobilization of **16** was calculated by the decrease

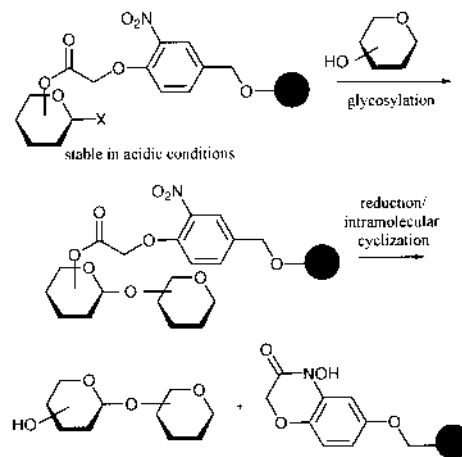


Chart 1

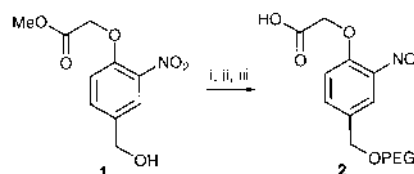


Chart 2

Table 1. Immobilization of Glycosyl Donor to Polymer Support

2+sugar unit (**3**, **4**, or **5**) \xrightarrow{i} polymer-supported glycosyl donor (**6**, **7**, or **8**)

Sugar unit	Product	Yield (%)
		Quant.
		94
		96

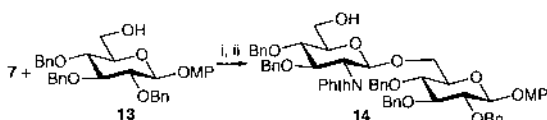
Reagents and conditions: i, sugar unit (2 eq), WSCDI, DMAP, CH₂Cl₂, r.t., overnight. Phth=Phtalimide.

* To whom correspondence should be addressed. e-mail: yukito@postman.riken.go.jp

Table 2. Reaction of Soluble Polymer-Supported Glycosyl Donor

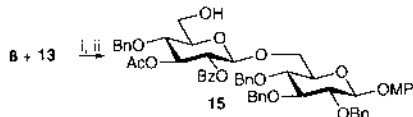
6 + acceptor $\xrightarrow{i, ii}$ product		
Acceptor	Product	Yield (%)
		87
		86

Reagents and conditions: i, DMTST, MS 3A, CH₂Cl₂, 0 °C—r.t., overnight; ii, Sn(SPh)₂, PhSH, Et₃N, PhH, r.t., overnight; MBz=4-methylbenzoyl, MP=4-methoxyphenyl.



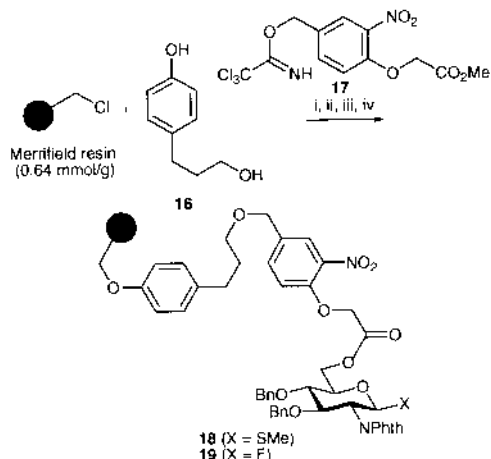
Reagent and conditions: i, Cp₂HfCl₂, AgOTf, MS 4A, CH₂Cl₂, 0 °C—r.t., overnight; ii, Sn(SPh)₂, PhSH, Et₃N, PhH, r.t., overnight, 82%.

Chart 3



Reagent and conditions: i, NIS, TESOTf, 0 °C—r.t., overnight; ii, Sn(SPh)₂, PhSH, Et₃N, PhH, r.t., overnight, 59% (2 steps).

Chart 4



Reagents and conditions: i, Cs₂CO₃, DME, 40 °C, 2 d; ii, **17**, BF₃·OEt₂, CH₂Cl₂, 0 °C, overnight; iii, aq. NaOH, THF, r.t., overnight; iv, **3** or **4**, WSCDI, HOBT, DMAP, r.t., overnight; or **3** or **4**, PPh₃, DEAD, THF, r.t., overnight.

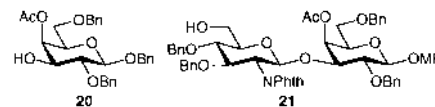
Chart 5

in chlorine content which correlated with the increase in the value for nitrogen, after reaction with **17**. Furthermore, the thioglycoside content was determined by the sulfur atom percentage. From the elemental analysis (S; 0.70%), the yield (from Merrifield resin to **18**, four steps) was calculated to be

Table 3. Reaction of Insoluble Resin-Immobilized Glycosyl Donor

18 or 19 $\xrightarrow{\text{glycosylation}}$		$\xrightarrow{\text{Sn(SPh)}_2, \text{PhSH}, \text{Et}_3\text{N}, \text{PhH}}$	product	
Donor	Acceptor	Glycosylation conditions	Product	Yield (%)
18	10	NIS, TESOTf CH ₂ Cl ₂ , r.t., 15 h	12	74 ^{a)} (38) ^{b)}
18 ^{c)}	20	NIS, TESOTf CH ₂ Cl ₂ , r.t., 16 h	21	66 ^{d)} (60) ^{b)}
19	20	Cp ₂ HfCl ₂ , AgOTf MS 4A, CH ₂ Cl ₂ 0 °C—r.t., 15 h	21	56 ^{a)} (29) ^{b)}

a) Based on **18** or **19** (0.33 mmol/g), b) overall yields from Merrifield resin (0.64 mmol/g), c) high loading **18** (thioglycoside content 0.58 mmol/g) was used, d) based on **18** (0.58 mmol).



52% (0.33 mmol/g), which was in close proximity to the value obtained after releasing thioglycoside **3** (NaOMe, MeOH, 51% overall yield).⁸⁾ The glycosylation of **18** with **10** was effected by DMTST, and disaccharide **12** was obtained in 74% yield (Table 3). Not only thioglycoside, but also fluoride **19** was activated under Suzuki conditions (Hf₂CpCl₂—AgOTf) to give disaccharide **21**.

In conclusion, the nitro-modified Wang resin-type linker proved to be useful for immobilizing glycosyl donors onto soluble and insoluble polymer supports. Throughout the reaction sequence, the linker was stable and cleavage can be performed under mild conditions without affecting commonly used protecting groups. As a result, direct use of the oligosaccharide products for block condensation can be considered.

Acknowledgments This work was supported by a Grant-in-Aid for Encouragement of Young Scientists from the Ministry of Education, Science, Sports and Culture, the Presidential Fund of RIKEN (S. M.), and the New Energy and Industrial Technology Development Organization. We thank Dr. T. Chihara and his staff for elemental analysis, and Ms. A. Takahashi for technical assistance.

References and Notes

- For recent reviews, see Ito Y., Manabe S., *Curr. Opin. Chem. Biol.*, **2**, 701—708 (1998); Seeberger P. H., Hasse W.-C., *Chem. Rev.*, **100**, 4349—4394 (2000).
- Manabe S., Nakahara Y., Ito Y., *Synlett*, **2000**, 1241—1244, and references therein.
- Melean L. G., Hasse W.-C., Seeberger P. H., *Tetrahedron Lett.*, **41**, 4329—4333 (2000); Tolborg J. F., Jensen K. J., *Chem. Commun.*, **2000**, 147—148; Wu X., Grathwohl M., Schmidt R. R., *Org. Lett.*, **3**, 747—750 (2001).
- Fügedi P., Garegg P. J., *Carbohydr. Res.*, **149**, c9—c12 (1986).
- Bartra M., Romea P., Urpi F., Vilarrasa J., *Tetrahedron*, **46**, 587—594 (1990).
- Hart G. W., Kreppel L. K., Comer F. I., Arnold C. S., Snow D. M., Ye Z., Cheng X., Dellamanna D., Caine D. S., Earles B. J., Akimoto Y., Cole R. N., Haynes B. K., *Glycobiology*, **6**, 711—716 (1996); Comer F. I., Hart G. W., *J. Biol. Chem.*, **275**, 29179—29182 (2000).
- Suzuki K., Maeta H., Matsumoto T., *Tetrahedron Lett.*, **30**, 4853—4856 (1989).
- It was possible to increase loading thioglycoside loading up to 0.58 mmol/g (calculated after cleavage; elemental analysis calculation for S; 1.22%) by repeating the addition reaction of phenol **16** to Merrifield resin twice.