ORGANIC LETTERS

XXXX
Vol. XX, No. XX
000–000

Stereoselective Ribosylation of Amino Acids

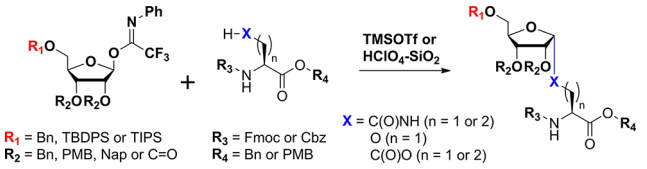
Hans A. V. Kistemaker, Gerbrand J. van der Heden van Noort, Herman S. Overkleeft, Gijsbert A. van der Marel,* and Dmitri V. Filippov*

Leiden Institute of Chemistry, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

marel_g@chem.leidenuniv.nl; filippov@chem.leidenuniv.nl

Received April 4, 2013

ABSTRACT



The glycosylation properties of ribofuranosyl *N*-phenyltrifluoroacetimidates toward carboxamide side chains of asparagine and glutamine were investigated. Conditions were found that promote nearly exclusive formation of the α-anomerically configured *N*-glycosides. The strategy allows for the synthesis of Fmoc-amino acids suitably modified for the preparation of ADP-ribosylated peptides. Furthermore, ribosylation of serine with these donors proved to be completely α-selective, and for the first time, α-ribosylated glutamic and aspartic acid, the naturally occurring sites for poly-ADP-ribosylation, were synthesized.

Glycosylation of proteins is a post-translational modification that plays a significant role in many biological processes.[1,2] Adenosine diphosphate ribosylation (ADPr) (Figure 1) is a peculiar type of protein glycosylation that occurs in both mono- and polymeric form and is considered to play an important role in a wide range of biological processes including cell proliferation, immune response, DNA repair, transcription regulation, and apoptosis.[3−6] The process of ADP-ribosylation requires the enzymatic transfer of a single ADP-ribose moiety from β-NAD$^+$ to the nucleophilic side chain of an amino acid forming an α-glycosidic linkage (dotted box, Figure 1). Enzymes (called PARPs) that are able to transfer additional ADP-ribosyl units to the 2′-OH of the adenosine moiety effect the formation of poly-ADP-ribose (Figure 1). The various identified sites amenable to ADP-ribosylation are asparagine, glutamic acid, aspartic acid, arginine, serine, and cysteine.[3,7] The construction of well-defined ADP-ribosylated peptides and analogues thereof would be of significant help in gaining a better understanding of the role and function of ADP-ribosylation.[8,9]

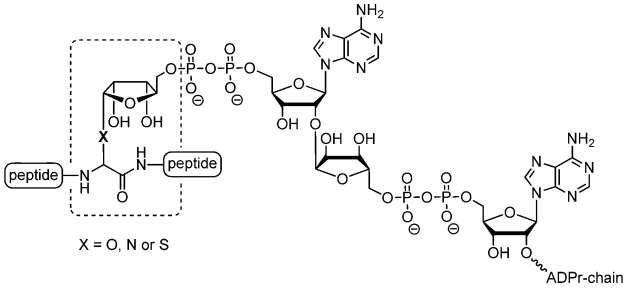


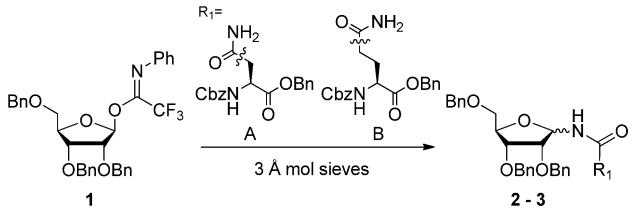
Figure 1. Poly-ADP-ribose polymer linked to a peptide.

One of the crucial steps in the synthesis of ADP-ribosylated peptides is construction of the α-glycosidic linkage

(1) Walsh, C. T.; Garneau-Tsodikova, S.; Gatto, G. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 7342.
(2) Khidekel, N.; Hsieh-Wilson, L. C. *Org. Biomol. Chem.* **2004**, *2*, 1.
(3) Hassa, P. O.; Haenni, S. S.; Elser, M.; Hottiger, M. O. *Microbiol. Mol. Biol. R.* **2006**, *70*, 789.
(4) Gibson, B. A.; Kraus, W. L. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 411.
(5) Schreiber, V.; Dantzer, F.; Ame, J. C.; de Murcia, G. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 517.
(6) Adams-Phillips, L.; Briggs, A. G.; Bent, A. F. *Plant. Physiol.* **2010**, *152*, 267.
(7) Cervanteslaurean, D.; Loflin, P. T.; Minter, D. E.; Jacobson, E. L.; Jacobson, M. K. *J. Biol. Chem.* **1995**, *270*, 7929.
(8) Lin, H. *Org. Biomol. Chem.* **2007**, *5*, 2541.
(9) Hengel, S. M.; Goodlett, D. R. *Int J. Mass. Spectrom.* **2012**, *312*, 114.

10.1021/ol400929c

Table 1. Ribosylation of Asparagine and Glutamine[a]



no.	acceptor	solvent	TMSOTf (equiv)	temp (°C)	yield (%)	ratio (α/β)[16]
1	A	CH_2Cl_2	0.2	−20 to rt	58	96/4
2	B	CH_2Cl_2	0.3	−78 to rt	38	87/13
3	B	CH_2Cl_2	0.2	−20 to rt	56	69/31
4	B	CH_3NO_2	0.3	0	67	77/23

[a] Reaction conditions: 1 equiv of acceptor (50 mM), 1.25 equivof donor, and 1.5 h reaction time.

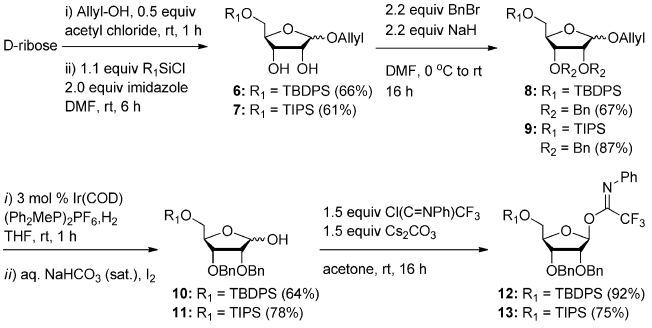
between the ribofuranosyl moiety and the amino acid side chain. We have reported the first fully synthetic approach to ADP-ribosylated peptides via the incorporation of an α-ribosyl containing glutamine or asparagine building block by solid-phase peptide synthesis (SPPS) followed by installment of the adenosine diphosphate linkage. These modified amino acids were prepared by us[10] and others[11] via reduction of ribofuranosyl azide and in situ condensation of the formed amine to the side chain of suitably protected glutamic acid or aspartic acid derivatives. The condensation products in this key amide bond formation were obtained as an anomeric mixture (75/25: α/β).[10]

We reasoned that the preparation of side-chain ribosylated amino acids might proceed with better α-selectivity when using acid-catalyzed glycosylation. Trifluoroacetimidate chemistry[12] was deemed to be the most viable for introducing the crucial α-linkage between the ribosyl moiety and the amino acids.[12−14] Our results in the use of ribofuranosyl trifluoroacetimidates in the α-selective construction of a series of ribosylated amino acids corresponding to the sites of ADP-ribosylation are reported here.

We decided to use donor **1**,[13] since it is known from literature[13,15] that nonparticipating benzyl protection on the ribofuranosyl donor predominantly renders the α-product in glycosylation reactions. Donor **1** was reacted with either *N*-Cbz- asparagine-OBn (acceptor A, Table 1) or *N*-Cbz-glutamine-OBn (acceptor B, Table 1) under various conditions. Ribosylation of asparagine using donor **1** and TMSOTf as activator gave an excellent α-selectivity (entry 1, Table 1). Application of identical conditions for glutamine resulted in a decrease in selectivity (entry 3, Table 1). Lowering the reaction temperature improved the α-selectivity but also reduced yield (entry 2, Table 1). Changing the solvent to nitromethane[14] (CH_3NO_2) did improve the yield but the α-selectivity remained poor (entry 4, Table 1).

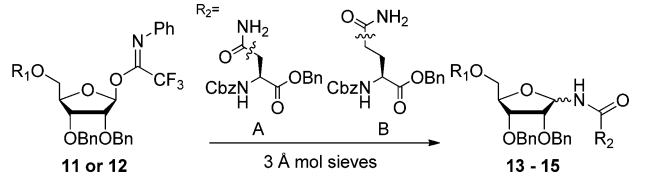
We anticipated that the selectivity could be improved by introducing a more bulky substituent at the 5′-position. For this reason, two new donors with *tert*-butyldiphenylsilyl (TBDPS), **11**, or triisopropylsilyl (TIPS) at the 5′-position, **12**, were synthesized (Scheme 1).

Scheme 1. Synthesis of 5′-Silylated Imidate Donors



To investigate the glycosylation properties of donor **11** and **12**, *N*-Cbz-glutamine-OBn was reacted with these under various conditions. The most favorable conditions for the ribosylation of glutamine turned out to be activation at −20 °C with 0.2 equiv of TMSOTf (entries 1 and 2, Table 2).[17] Using either of the two 5′-silylated donors, **11** and **12**, greatly improved the α-selectivity compared to the use of 5′-benzylated donor **1** (entry 2, Table 1). Ribosylation of asparagine (A) with TIPS-donor **12** still gave a higher α-selectivity than that of glutamine (B) although the difference in selectivity was greatly reduced compared to the tri-*O*-benzyl donor (entry 3, Table 2). For these

Table 2. Optimized Ribosylation of Glutamine and Asparagine[a]

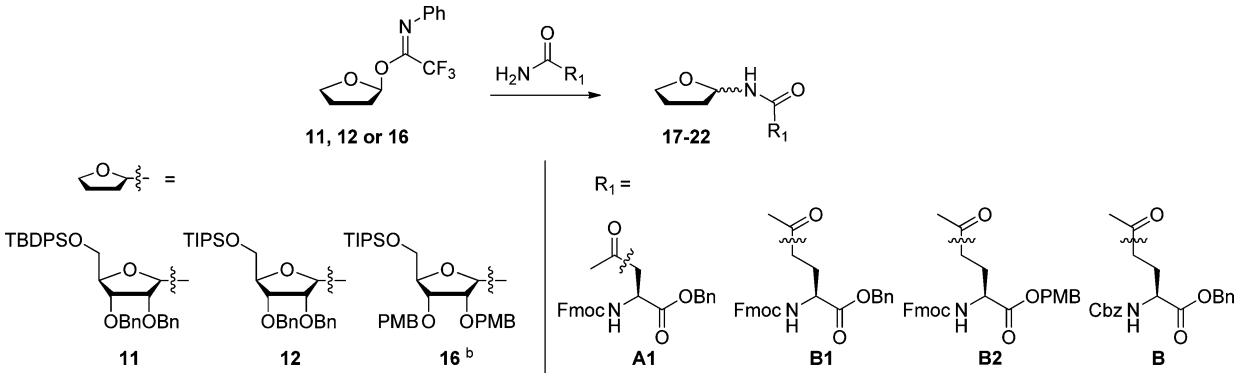


no.	acceptor	donor (R_1=)	product	temp (°C)	yield (%)	ratio (α/β)
1	B	**11** (TBDPS)	13	−20 to rt	57	95/5
2	B	**12** (TIPS)	14	−20 to rt	66	95/5
3	A	**12** (TIPS)	15	−20 to rt	68	98/2

[a] Reaction conditions: 1 equiv of acceptor (50 mM), 1.25−1.5 equiv of donor, 0.2 equiv of TMSOTf, DCM, and 1.5 h reaction time.

(10) van Noort, G. J. V.; van der Horst, M. G.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V. *J. Am. Chem. Soc.* **2010**, *132*, 5236.
(11) Bonache, M. A.; Nuti, F.; Le Chevalier Isaad, A.; Real-Fernández, F.; Chelli, M.; Rovero, P.; Papini, A. M. *Tetrahedron Lett.* **2009**, *50*, 4151.
(12) Yu, B.; Tao, H. C. *Tetrahedron Lett.* **2001**, *42*, 2405.
(13) van Noort, G. J. V.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V. *Org. Lett.* **2011**, *13*, 2920.
(14) Tanaka, H.; Iwata, Y.; Takahashi, D.; Adachi, M.; Takahashi, T. *J. Am. Chem. Soc.* **2005**, *127*, 1630.
(15) Larsen, C. H.; Ridgway, B. H.; Shaw, J. T.; Smith, D. M.; Woerpel, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 10879.

Table 3. Ribosylation of Fmoc-asparagine and Fmoc-glutamine Benzyl Esters[a]



no.	donor	acceptor	product	activator	solvent	temp (°C)	yield (%)	ratio (α/β)
1	**11**	A1	**17**	TMSOTf	dioxane/DCM	0	44	97/3
2	**11**	A1	**17**	TMSOTf	dioxane/DCM	rt	63	93/7
3	**11**	B1	**18**	TMSOTf	dioxane/DCM	0	49	72/28
4	**11**	B2	**19**	TMSOTf	dioxane/DCM	0	46	73/27
5	**12**	B1	**20**	$HClO_4$–SiO_2	dioxane/DCM	rt	59	96/4
6	**16**	B	**21**	TMSOTf	DCM	−20 to rt	52	85/15
7	**16**	B1	**22**	TMSOTf	dioxane/DCM	−20 to rt	60	78/22
8	**16**	B1	**22**	$HClO_4$–SiO_2	dioxane/DCM	rt	69	93/7

[a] Reaction conditions: 1 equiv of acceptor (50 mM), 1.5 equiv of donor, 0.2 equiv pf activator, and 1.5 h reaction time. [b] For the synthesis of donor **16**, see the Supporting Information.

ribosylated amino acids to be useful in SPPS, protective group exchange was required and was exemplified for compounds **14** and **15** (see the Supporting Information).

It occurred to us, at this stage, that direct ribosylation of Fmoc-amino acids would give a more straightforward entry to the target building blocks. The feasibility of this approach was first tested in the ribosylation of *N*-Fmoc-asparagine-OBn with donor **11**. Because of the poor solubility of the Fmoc-amino acid, the solvent system had to be changed to methylene chloride/dioxane (1:1, v/v). The ribosylation went almost completely α-selective but in a moderate yield when performed at 0 °C (entry 1, Table 3), whereas at room temperature the stereoselectivity only slightly decreased while the yield significantly increased (entry 2, Table 3). The yield and selectivity are comparable with respect to the *N*-Cbz-protected asparagine derivative, and this approach circumvents tedious protecting group manipulations. However, these promising results were not reflected in the ribosylation of *N*-Fmoc-glutamine-OBn, which showed a modest excess of the α-anomer and an average yield at 0 °C (entry 3, Table 3). Replacing the benzyl ester with 4-methoxybenzyl (PMB), in an attempt to improve the solubility of the amino acid, did not improve the outcome of the reaction in any way (entry 4, Table 3). Therefore, we investigated the use of perchloric acid on silica ($HClO_4$–SiO_2) as activator, for it has been used with glycosylations in the presence of dioxane at room temperature.[18,19] The $HClO_4$–SiO_2 reagent was prepared according to literature procedure[20] and added to a mixture of *N*-Fmoc-glutamine-OBn and donor **12** at room temperature. The stereoselectivity increased tremendously along with a good yield (entry 5, Table 3).

The hydrogenolysis of benzyl ethers at the ribosyl moiety was found to be incompatible with the integrity of the Fmoc protection. Therefore, we introduced the acid sensitive PMB at the 2′- and 3′-position (donor **16**, Table 3). The donor proved to be a little less α-selective in the ribosylation reaction of *N*-Cbz-glutamine-OBn (entry 6, Table 3) compared to the benzylated donor **12** (95/5 vs 85/15 α/β). Ribosylation of *N*-Fmoc-glutamine-OBn with donor **16** in the presence of TMSOTf showed a comparable yield and α-selectivity (entry 7, Table 3) to benzylated donor **11** (entry 3, Table 3), which could be again increased significantly by activation with $HClO_4$–SiO_2 (entry 8, Table 3).

This optimized strategy allows for the synthesis of suitable protected glutamine building blocks for SPPS in a straightforward approach and at a preparative scale (0.5 mmol). This is demonstrated for compound **22**, which was transformed in three consecutive steps into compound **23** (Figure 2).[21]

The results of the glycosylation experiments encouraged us to test the three donors (**1**, **11**, and **12**) on other amino acid side chains. The anomeric ratio for the ribosylation of serine (Ser, Table 4) with tribenzyl donor **1** (entry 1, Table 4) was comparable to glutamine (entries 1 and 2, Table 1) and to the reported data.[22,23] Ribosylation with the 5′-silylated donors, **11** and **12**, on the other hand gave exclusively α-ribosylated serine (entry 2 and 3, Table 4). To our knowledge, this is the first example of a completely α-selective ribosylation for an amino acid.

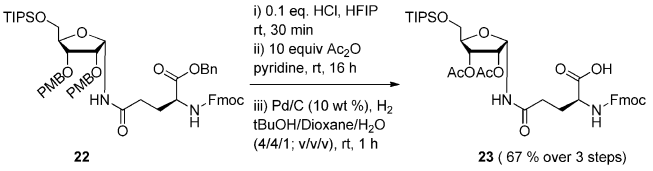


Figure 2. Synthesis of Fmoc Building Block **23** Suitable for SPPS.

Next, glutamic acid and aspartic acid were subjected to ribosylation with donor **12**.[24] The stereoselectivity for both amino acids was mostly lost and in the best case a small excess of α-ribosylated glutamic acid (77/23 α/β) was obtained (entry 4, Table 4). Lowering the temperature did not improve the stereochemical outcome of both reactions and the best result for aspartic acid was a 50:50 mixture of α/β-anomers (entry 6, Table 4). Moreover, using $HClO_4$–SiO_2 as activator did not improve the outcome of the ribosylations (entries 5 and 7, Table 4). Nevertheless, both anomers could easily be separated by silica gel chromatography at this stage to afford novel α-ribosylated glutamic acid and aspartic acid derivatives.

This first successful attempt to obtain α-ribosylated glutamic acid ever reported might lead to ADP- ribosylated peptides with glutamic acid,for example those derived from histone H2B.[3] So far, ribosylated glutamine is used as an isostere of glutamic acid in the synthesis of such peptides.[10]

(16) The anomeric ratio of the glycosylation products was determined by ^{1}H NMR, and the individual anomers could be assigned by HECADE-NMR spectroscopy: Napolitano, J. G.; Gavín, J. A.; García, C.; Norte, M.; Fernández, J. J.; Hernández Daranas, A. *Chem.—Eur. J.* **2011**, *17*, 6338.

(17) Although lower temperatures (∼−50 °C) resulted in highly α-selective ribosylations (>98% α), yields were fairly low (<20%). Silylation of glutamine, before ribosylation, with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was employed in order to increase the solubility, but this did not improve the solubility or yield. Replacing the benzyl ethers with 2-naphthylmethyl or cyclic carbonate at the 2′- and 3′-positions improved neither the yield nor selectivity.

(18) Mukhopadhyay, B.; Maurer, S. V.; Rudolph, N.; van Well, R. M.; Russell, D. A.; Field, R. A. *J. Org. Chem.* **2005**, *70*, 9059.

(19) Ludek, O. R.; Gu, W. L.; Gildersleeve, J. C. *Carbohydr. Res.* **2010**, *345*, 2074.

(20) Immobilized perchloric acid on silica was prepared by adding $HClO_4$ (2 mmol, as a 70% aqueous solution) to a slurry of silica gel (5 g, 200 mesh) in Et_2O (15 mL) and was stirred for 1 h at room temperature. The solvent was removed under reduced pressure, and the resulting powder was dried at 110 °C for 2 h under reduced pressure to obtain $HClO_4$–SiO_2 (0.4 mmol H^+/g) and was used directly: Agarwal, A.; Rani, S.; Vankar, Y. D. *J. Org. Chem.* **2004**, *69*, 6137.

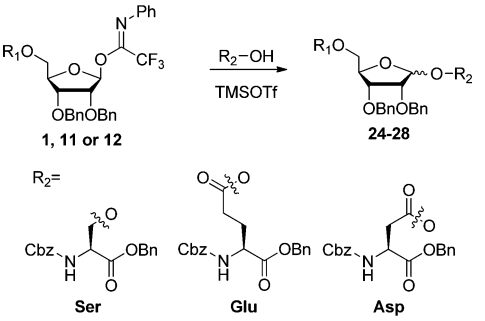
(21) The use of HFIP as the solvent and a catalytic amount of HCl in the acidolytic cleavage of the PMB ethers proved to be essential for preserving the configuration of the anomeric center. For application of HFIP as a solvent in acidolysis of protective groups, see: Palladino, P.; Stetsenko, D. A. *Org. Lett.* **2012**, *14*, 6346.

(22) Suda, S.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 1211.

(23) Gravier-Pelletier, C.; Ginisty, M.; Le Merrer, Y. *Tetrahedron: Asymmetry* **2004**, *15*, 189.

(24) The use of donor **16** in the ribosylation of glutamic- and aspartic acid showed spontaneous and nonstereoselective product formation without the addition of activator.

Table 4. Ribosylation of Serine, Glutamic Acid, and Aspartic Acid[a]



no.	donor (R_1 =)	acceptor (R_2)	product	temp (°C)	yield (%)	ratio (α/β)
1	Bn	Ser	24	−50	78	75/25
2	TBDPS	Ser	25	−50	60	100/0
3	TIPS	Ser	26	−50	56	100/0
4	TIPS	Glu	27	−50	84	77/23
5	TIPS	Glu	27	−50	73	60/40[b]
6	TIPS	Asp	28	−70	63	50/50
7	TIPS	Asp	28	−78	74	43/57[b]

[a] Reaction conditions: 1 equiv of acceptor (50 mM), 1.25 equiv of donor, 0.2 equiv of TMSOTf. [b] 0.05 equiv of $HClO_4$–SiO_2, DCM for 1.5 h.

In conclusion, we have presented a study on the preparation of various ribosylated amino acids in a highly α-selective fashion by tuning the protective groups at the 2′-, 3′-, and 5′-position of the ribofuranosyl imidate donor. Furthermore, the number of synthetic steps toward SPPS building blocks was minimized by using Fmoc protected amino acids, simplifying the protective group manipulation strategy. This new protocol allows for the synthesis of ribosylated Fmoc building blocks in nine steps starting from D-ribose at a preparative scale. In the specific case of glutamine, the glycosylation reaction using $HClO_4$–SiO_2 as activator proved to offer great improvement compared to TMSOTf, with respect to α-selectivity. Finally, we showed a completely α-selective ribosylation and prepared for the first time ribosylated aspartic and glutamic acid using the trfluoroacetimidate donors. Our method can conceivably be extended to the ribosylation of other amino acids.

Acknowledgment. The Netherlands Organization for Scientific Research (NWO) is acknowledged for financial support.

Supporting Information Available. Spectroscopic data and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.