Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

A new approach for the synthesis of *O*-glycopeptides through a combination of solid-phase glycosylation and fluorous tagging chemistry(SHGPFT)

Bo Liu,[a] Fa Zhang,[a] Yan Zhang, [a] Gang Liu*[a,b,c]

Received (in XXX, XXX) XthXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Glycoproteins and glycopeptides play important roles in various physiological and pathophysiological processes. Efficient preparation of glycopeptides with a specific structure is one of the pivotal areas in current chemistry research. In this article, a new SHGPFT approach to the synthesis and efficient purification of *O*-glycosylated peptides is developed by combining a solid-phase glycosylation synthesis and a light-fluorous glycosyl donor protocol. The desired product is finally isolated from the side products in the cleaved mixture by an efficient fluorous solid-phase extraction (F-SPE) step.

Glycoproteins and glycopeptides play vital roles in physiological processes, such as cell adhesion, cell differentiation, and cell growth.[1] Most of theapproved protein-based drugs, representing a quarter of new drugs, are glycoproteins.[2] However, progress toward understanding the functions of glycoproteins and glycopeptides and analyzing their structure–activity relationships (SARs) is restricted by their limited supplemental resources. This includes difficult separation from natural glycoforms because of their microheterogenicity at carbohydrate portions[3] and uncontrollable glycosylation in the biosynthetic approach.[4] To overcome these obstacles, there is a need for rapid and efficiently synthetic approaches to access the target glycopeptides, especially methods for preparing pure and structurally well-defined glycopeptides libraries for vaccine development and drug discovery.

Solid-phase peptide synthesis (SPPS), originally developed by Merrifield,[5] has been well applied in recent years by the introduction of automation and progressive methodologies,[6] however, efficient construction of glycopeptides via SPPS is still challenging because of the complexity of hybrid peptide assembly and oligosaccharide growth plus the difficult purification of the final glycopeptide. Using preformed glycosylated amino acids or oligosaccharides as building blocks during solid-phase glycopeptide synthesis is currently the most popular method for synthesis of glycopeptides,[7] while it suffers from cumbersome operation, low efficiency, and an apparently variable synthetic outcome from one target product to another.[8] Thus, it is an effort-saving strategy to directly access the carbohydrate portion onto the solid support to assemble the glycopeptides.[9]

To directly assemble carbohydrate on the solid phase, the choice of a proper resin and linker is important. Our group has found that the use of a large excess of Lewis acid can lead to glycosylation of the free hydroxyl group of a single amino acid on TentaGel resin, which is one of the most popular resins used for solid-phase organic synthesis and on-bead screening.[10] However, the presence of the large excess of Lewis acid restricts the use of acid-liable linkers. On the other hand, strong basic conditions for cleavage of a base-sensitive linker could lead to β-elimination of glycopeptides and could also isomerize the stereogenic centers of peptides.[11] Consequently, we decided to employ an aryl hydrazine safety-catch linker[12] for a hybrid glycopeptides synthesis.

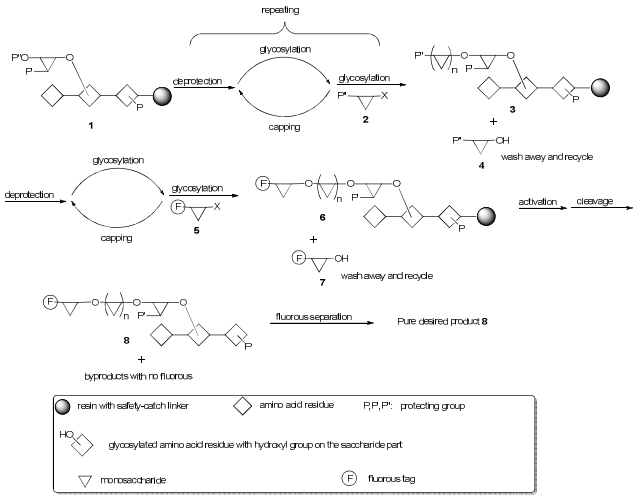
The isolation of glycopeptides away from chromatographically similar impurities (this commonly means a deletion sequence on the saccharide segment or truncated peptides) is also a problematic, time-consuming, high-cost, and low-yield step. Fluorous chemistry has been widely used in biphasic catalysis, synthesis, and separation of small organic molecules and biomolecules,[13] for instance, peptides,[14] oligosaccharides,[15] and oligonucleotides.[16] However, it has never been used in the synthesis of glycopeptides on solid supports. Employment of fluorous chemistry on solid-phase synthesis of glycopeptides should give all the advantages of both solid-phase synthesis and fluorous chemistry. Obviously, this approach will be very complicated because it integrates peptide chemistry, solid-phase synthesis, carbohydrate chemistry, and fluorous chemistry.

Herein, we report the first attempt of solid-phase synthesis of *O*-linked glycopeptides through the combination of a solid-phase hybrid glycopeptidation procedure and fluorous tagging method (SHGPFT) (Scheme 1). In the SHGPFT glycosylation steps, the deprotection of a temporary protected saccharide **1** on the resin to afford an acceptor is followed by the glycosylation with a

[a]*Institute of MateriaMedica, Chinese Academy of Medical Sciences & Peking Union Medical College, 2 Nanwei Rd., Xicheng Dist., Beijing 100050, P. R. China.*[b]*Tsinghua-Peking Center for Life Sciences and* [c]*Department of Pharmacology and Pharmaceutical Sciences, School of Medicine, Tsinghua University, Haidian Dist., Beijing 100084, P. R. China. Fax: +86 010 63167165;Tel: +86 010 63167165*
E-mail: gangliu27@biomed.tsinghua.edu.cn

† Electronic Supplementary Information (ESI) available: General methods, Experimental procedure, Spectroscopic data of products. See DOI: 10.1039/b000000x/

nonfluorousglycosyl donor **2**. The cycle is repeated to build the anticipated oligosaccharide moiety **3** on the resin. This solid-phase synthesis procedure has the advantage of simply washing

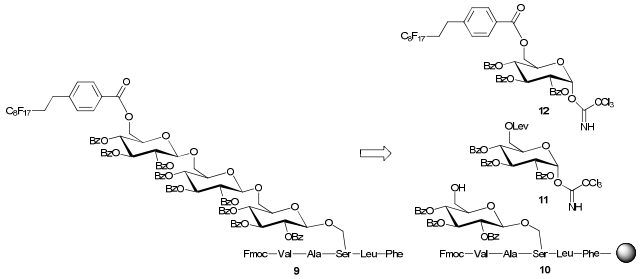


Scheme 1 Schematic overview of our SHGPFT approach for a hybrid glycopeptides synthesis

away all unreacted reagents such as the catalyst of the solid-phase glycosylation and the inactive monosaccharide **4** from the recycling of excessive donor **2**. In the last cycle, a light-fluorous tagged glycosyl donor **5**, which was developed by our group,[17] was introduced to give **6**. As a result, the desired glycopeptide **8** with a fluorous tag was afforded as well as truncated sequences without the fluorous tag on the resin and with other inclusions such as the fluorous monosaccharide **7** in the solution. Following simple filtration and cleavage of the glycopeptides under mild conditions through an aryl hydrazine linker, the designed pure product **8** was obtained simply by fluorous solid-phase extraction (F-SPE).

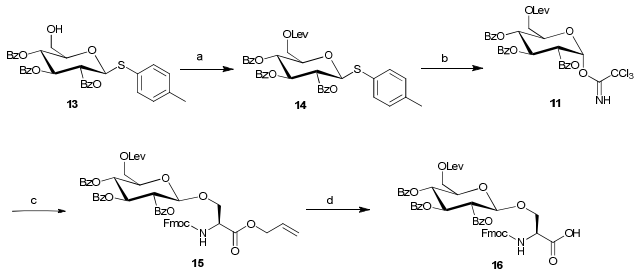
There are several beneficial aspects of this SHGPFT method. First, the incomplete coupling of a glycosyl-amino acid to the growing peptide chain on the solid support generally induces large amounts of impurity.[8] This is always the major reason for sequence deficiency of the peptide motif in the preparation of glycopeptides. Purification of the desired product from the deletion sequence is time-consuming. This SHGPFT protocol enormously simplifies the purification procedure by means of the F-tag of the fluorous phase. Second, the stable aryl hydrazine linker under reactions conditions eliminates the risk of destroying the structure of the glycopeptide because of its acidic and/or basic liability to the glycomoiety and side-protection groups of peptides. Third, no matter how structurally variable and complex the desired glycopeptides are, only the F-taggedglycosyl donor in the final assembly step requires special preparation, so this approach is applicable to a wide scope of glycopeptide synthesis. Moreover, each component of the saccharide portion and/or the peptide motif could be diversified with other homologous building blocks; therefore, this SHGPFT method provides the possibility of efficient construction of a pure glycopeptide library with determinate structures. Finally, because only the final target glycopeptides(s) contains the F-tag, the pure desired product can be obtained after a simple F-SPE, even if the solid-phase synthetic outcome is not perfect.

To test this SHGPFT method, the glycopeptide **9** was employed as the desired molecule, in which the Ser is placed in the middle of the peptide sequence to simulate as closely as possible to the glycosylated site in natural glycopeptides (Scheme 2). To complete the synthesis of **9**, three key building blocks were pre-synthesized (Scheme 2) including the pentapeptide that contains an *O*-linked saccharide moiety on the resin **10**; the non-fluorous tagged glycosyl donor **11**; and the fluorous tagged glycosyl donor **12**. The monosaccharide **11** was designed to elongate the carbohydrate chains for the route. The temporary protecting Lev group is suitable to be removed on this designed solid support with a employed aryl hydrazine linker. All the glycosyl donors were transformed into a trichloroacetimidate form because of its ideal properties for solid-phase glycosylation.[18]



Scheme 2 Retrosynthesis of our target pentapeptides with trisaccharide

Glucopyranosyl trichloroacetimidate**11** was prepared from **13** after introduction of the Lev group and transformation of the thioglycoside. After glycosylation of Fmoc-Ser-OAll[19] with **11**, followed by $Pd(PPh_3)_4$-catalyzed deprotection of the allyl group, **16** was obtained(Shceme 3).The preparation of compound **10** with the aryl hydrazine linker was finished by the prepared glycosylSer**16** with a standard SPPS protocol (Scheme 4).

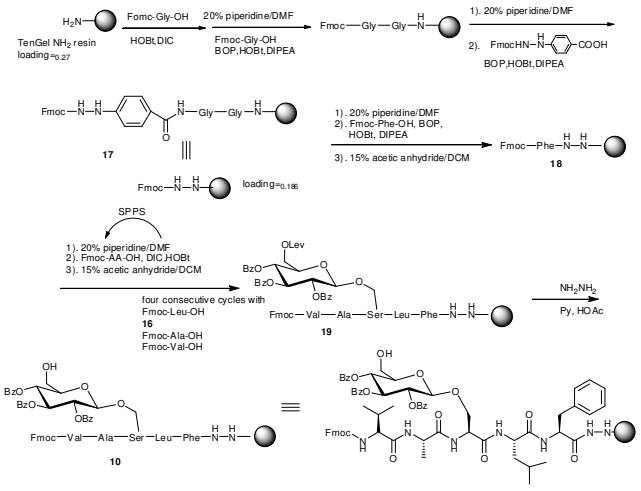


Scheme 3 a) LevOH, DCC, DMAP,DCM 92%; b) i. NBS,TMSOTf,H_2O, DCM; ii. Cl_3CCN, DBU, DCM, 0 °C, two-step yield 62%; c) Fmoc-Ser-OAll, TMSOTf, DCM, 0 °C, 4Å MS, 64%; d) $Pd(PPh_3)_4$, morpholine, DCM 96%

The peptide chain assembly was achieved by using of a Gly–Gly spacer functionalizingTentaGel amino resin, which was further equipped with an aryl hydrazine linker (loading capacity: 0.186mmol/g). The assembly of the peptide chain was performed according to a standard Fmoc-based protocol. Loading of the first amino acid (Phe) was realized with a BOP/HOBt/DIPEA cocktail of reagents to make the condensation complete, while the standard DIC/HOBt protocol was used to extend the other peptide chain. After each incorporation of a Fmoc-protected amino acid, the reaction support was treated with Ac_2O in DCM (15%) to cap

the trace unreacted amino group. With the protected pentapeptide with monoglucose **19** in hand, the Lev group on the carbohydrate motif was selectively removed by a solution of N_2H_4/Py/HOAc that resulted in **10** as the glycosyl acceptor on the solid support (Scheme 4).

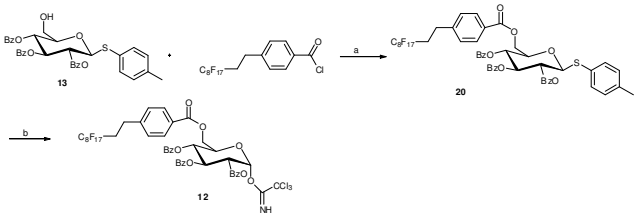
The solid-phase *O*-glycosylation of **10** was then performed under the optimal condition (10 equiv acceptor in the



Scheme 4 Solid-phase synthesis of pentapeptide with monosaccharide motif

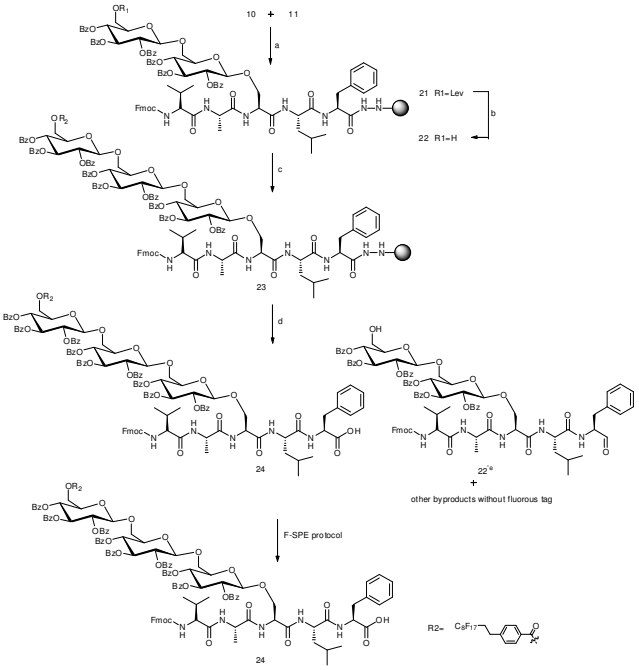
presence of 20 equiv TMSOTf) in advance to offer **21**. This condition did not affect the linker or any protecting groups. After the glycosylation, the reaction mixture was quenched with Et_3N, then the excess of the donor in the residue was recovered in the form of anomeric hydroxy glucose (86%). The unreacted free hydroxyl on the first saccharide fragment was then capped using acetic anhydride in pyridine (1:3=v:v). Subsequently, by removal of the Lev group of **21** under the above conditions, a second acceptor was assembled onto the solid support, i.e., a pentapeptide with disaccharide **22** on the resin was successfully obtained (Scheme 6).

To complete this new strategy, we required a fluorous glycosylated donor at the end of the carbohydrate chain elongation. The preparation of fluorous donor **12** was achieved from thioglycoside **13** by installation of a fluorous-tagged benzoyl chloride[17b] and transformation of thioglycoside **20** into a trichloroacetimidate **12** (Scheme 5).



Scheme 5 a) DMAP, Et_3N, DCM 90%; b) i. NBS, TMSOTf, H_2O, DCM; ii. Cl_3CCN, DBU, DCM, 0 °C; two-step yield 57%

After that, the pentapeptide with disaccharide **22** on the resin was reacted with fluorous donor **12** by the solid-phase glycosylation protocol used before to provide resin **23** (Scheme 6). The final product was released from the resin **23** in a two-step protocol, using *N*-bromosuccinimide as the oxidizing agent and subsequent addition of H_2O (5%) in THF solution. The crude mixture was analyzed by HPLC (Figure 1a). The desired



Scheme 6 Solid-phase glycosylation and F-SPE purified the cleavage residue: a) i. TMSOTf, 0 °C, DCM; ii. Ac_2O/Py=1:3, 1h b)NH_2NH_2, Py/AcOH; c) **12**, TMSOTf, 0 °C, DCM; d) i. NBS/Py, 15min; ii. H_2O, THF,4h; e) **22'** is the cleavage product of the resin **22**.

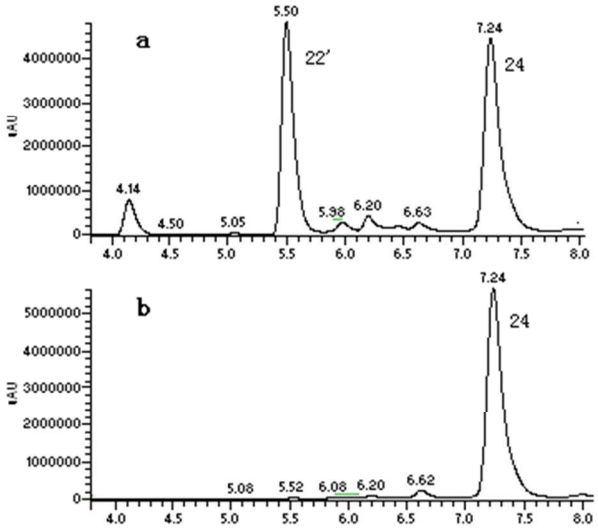


Figure 1 HPLC analysis profiles of glycosylation of **12** with **22**: a) crude product and b) fluorous fraction after F-SPE.

protected glycopeptide **24** was detectable along with the unglycosylated non-fluoride material **22'** and minor amounts of side products. We herein leave an incomplete coupling result in reaction step c in scheme 6 of **12** for one time assembly (Figure 1a) because we want to demonstrate the separating efficacy by F-

SPE. The yield of **24** after executing F-SPE protocol of the crude cleavage is 37% (one-step purification: see ESI†). In fact, repeated step c in scheme 6 of **12** assembly increased the yield of **24** (data did not show).

Notably, the recyclable fluorous glycosyl donor and fluorous silica gel used in the whole procedure avoided unnecessary costs for purification. Analysis of the fluorous fraction of F-SPE by HPLC (Figure 1b) indicated good purity (96%).

Conclusions

A new and efficient hybrid strategy to synthesize and purify *O*-glycopeptides was developed via a combination of the advantages of solid-phase glycosylation strategy with the superiority of fluorous chemistry. This approach not only introduces a fluorous-tagged glycosyl donor and aryl hydrazine safety-catch linker into the solid-phase synthesis of *O*-linked glycopeptides for the first time, but also avoids generation of extra costs with the help of solid-phase glycosylation and F-SPE chemical recycling. This SHGPFT strategy will be a significant advance in realizing automated *O*-linked glycopeptide synthesis for nonspecialists. The synthesis of more complex natural bioactive *O*-linked glycopeptides and construction of glycopeptide libraries for new active material discovery by our protocol is now in progress.

Acknowledgment

This research is supported financially by the National 863Program of China,No. 201202AA0303.

Notes and references

1 (a) H. Lis, N.Sharon, *Eur. J. Biochem.,* 1993, **218**, 1; (b) R.Dwek, *Chem. Rev.,* 1996, **96**, 683;(c) C. R.Bertozzi,L. L.Kiessling,*Science,*2001,**291**, 2357; (d)D. P. Gamblin, E. M. Scanlan and B. G. Davis,*Chem. Rev*., 2009, **109**, 131–163

2 T. J. Boltje,T.Buskas,G.-J. Boons,*Nature Chemistry* 2009,**3**4, 611.

3 P.M. Rudd, R. A.Dwek, *Crit. Rev. Biochem. Mol. Biol.*1997, **32**, 1.

4 R. P. Matthew and C. R. Bertozzi,*Chem. Soc. Rev.*, 2005, **34**, 58.

5 R. B. Merrifield, *J. Am. Chem. Soc.*,1963, **85**, 2149.

6 (a)P. Llovd-Williams, F. Albericio, E.Giralt, *Chemical Approaches to the Synthesis of Peptides and Proteins*.,(eds.) (1997) CRC LLC, NY; (b)T. Conroy, K. A. Jolliffe,R. J. Payne,*Org. Biomol. Chem.*, 2009, **7**, 2255; (c) M. Amblard, J.-A. Fehrentz, J. Martinez, and G. Subra, *Mol. Biotechnol.,*2006, **33**, 239.

7 (a) T. Reipen, H. Kunz *Synthesis,* 2003, 16,2487; (b) C. Brocke,H. Kunz, *Synlett,*2003, **13**, 2052; (c) M. A.Walczak, J. Hayashida, S. J. Danishefsky, *J. Am. Chem. Soc.,* 2013, **135,** 4700; (d) N.Yamamoto, A.Takayanagi, A. Yoshino,T.Sakakibara,Y.Kajihara, *Chemistry - A European Journal,* 2007, **13**, 613; (e)N. Shao, J. Xue, Z. Guo, *J. Org. Chem.,* 2003, **68**, 9003; (f)N. Shao, Z. Guo, *Org. Lett.* ,2005,**7**, 3589.

8 Y. Zhang, S. M. Muthana, D. Farnsworth, O. Ludek, K. Adams, J. J. Jr. Barchi, J. C.Gildersleeve, *J. Am. Chem. Soc.,* 2012, **134**, 6316

9 (a) M. Hollósi, E.Kollát, I. Laxzkó, K. F. Medzihradszky, J. Thurin,L. Jr.Otvös, *Tetrahedron Lett.,* 1991, **32**, 1531; (b) M. Meldal, F.-I. Auzanneau, O. Hindsgaul, M. M. Palcic, *J. Chem. Soc., Chem. Commun.* 1994, **16**, 1849; (c) H. Paulsen, A. Schleyer, N. Mathieux, M. Meldal, K. J. Bock, *Chem. Soc., Perkin Trans.1* 1997,**6**, 281; (d) A.Schleyer, M. Meldal, R. Manat, H. Paulsen, K. Bock, *Angew. Chem., Int. Ed.,* 1997, **36**, 1976; (e) K. M. Halkes, C. H. Gotfredsen, M.Grøtli, L. P. Miranda, J. O. Duus, M. Meldal, *Chem. Eur. J.,* 2001, **7**, 3584; (f) M. Mogemark, M. Elofsson, J. Kihlberg, *Org. Lett.* 2001, **3**, 1463; (g) M. Mogemark, F. Gårdmo, T. Tengel,J. Kihlberg, M. Elofsson, *Org. Biomol. Chem.* 2004, **2**, 1770.

10 N.-H.Yao, W.-Y. He, K. S.Lam, G.Liu, *J. Comb. Chem.* 2004, **6**, 214.

11 T. Buskas, S. Ingale, G.-J. Boons, Glycobiology 2006 16. 113R.

12 (a) C. R. Millington, R. Quarrell, G. Lowe, *Tetrahedron Lett.,* 1998, **39**, 7201; (b) C. Peters,; H. Waldmann, *J. Org. Chem.,* 2003, **68**, 6053; (c) Y. Kwon, K. Welsh, A. R. Mitchell, J. A. Camarero, *Org. Lett.,*2004, **6**, 3801; (d) J. A. Camarero, B. J. Hackel, J. J. de Yoreo, A. R. Mitchell, *J. Org. Chem*., 2004, 69, 4145; (e) A. Shigenaga, J. A. Moss, F. T. Ashley, G. F. Kaufmann, K. D. Janda, *Synlett,* 2006, **4**, 551.

13 (a) W. Zhang, *Chem. Rev.,*2004, **104**, 2531; (b) D. P. Curran, *Science,* 2008, **321**, 1645; (d) W. Zhang, *Chem. Rev.* 2009, **109**, 749 and reference therein.

14 P. C. de Visser, M. van Helden, D. V. Filippov, G. A.van der Marel, J. W. Drijfhout, J. H. van Boom, D. Noort, H. S. Overkleeft, *Tetrahedron Lett.* 2003, **44**, 9013.

15 (a) E. R. Palmacci, M. C. Hewitt, P. H. Seeberger, *Angew.Chem., Int.Ed.*, 2001, **40**, 4433; (b) F. R. Carrel,; P. H. Seeberger, *J. Org. Chem.* 2007, **73**, 2058.

16 (a) S. Tripathi, K. Misra, Y. S. Sanghvi, *Org. Prep. Proc*. 2005, 37, 257; (b) W. H. Pearson, D. A. Berry, P.Stoy, K.-Y. Jung, A. D. Sercel, *J.Org. Chem.* 2005, 70, 7114.

17 (a) F. Zhang, W. Zhang, Y. Zhang, D. P. Curran, G. Liu, *J.Org.Chem.* 2009, 74, 2594. (b) Y. Zhang, B. Liu,G. Liu, Mol Divers 2013, 17, 613.

18 (a) L. Schofield, M. C. Hewitt, K. Evans, M. A. Siomos, P. H. Seeberger, *Nature*, 2002, 418, 785; (b) P. H. Seeberger, C*hem. Soc. Rev*.,2008, 37, 19.

19 T. Vorherr ,W. Bannwarth, *Bioorg. Med. Chem. Lett.*, 1995, 5, 2661.