DOI: 10.1002/adsc.200700058

An Enzyme Module System for *in situ* Regeneration of Deoxythymidine 5′-Diphosphate (dTDP)-Activated Deoxy Sugars

Carsten Rupprath,[a] Maren Kopp,[b] Dennis Hirtz,[a] Rolf Müller,[b,*] and Lothar Elling[a,*]

[a] Laboratory for Biomaterials, Institute of Biotechnology and Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Worringer Weg 1, 52074 Aachen, Germany
Fax: (+49)-241-80-22387; e-mail: l.elling@biotec.rwth-aachen.de
[b] Saarland University, Department of Pharmaceutical Biotechnology, P. O. Box 151150, 66041 Saarbrücken, Germany
Fax: (+49)-681-302-5473; e-mail: rom@mx.uni-saarland.de

Received: January 24, 2007; Revised: April 6, 2007

Dedicated to Prof. Dr. Dr. h.c. Maria-Regina Kula on the occasion of her 70th birthday (16.03.2007).

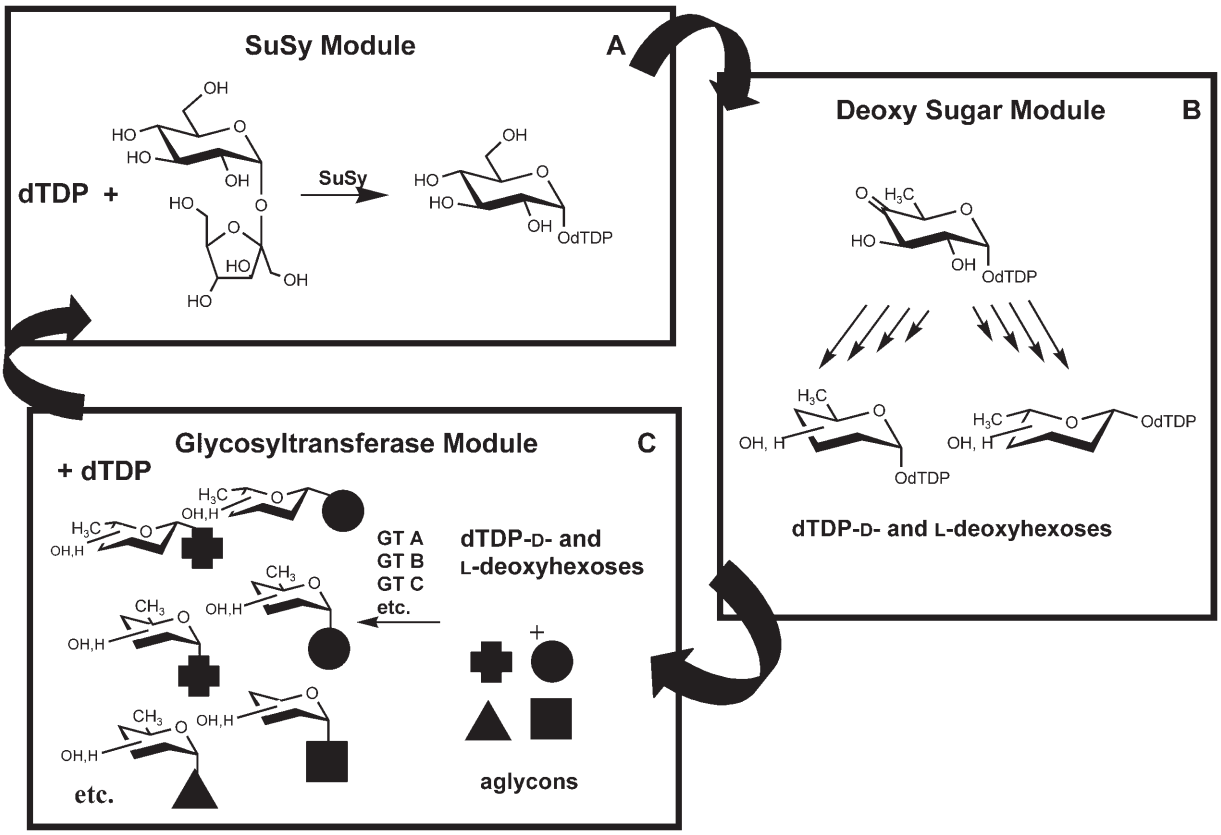
Supporting information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

Abstract: A highly flexible enzyme module system (EMS) was developed which allows for the first time the *in situ* regeneration of deoxythymidine 5′-diphosphate (dTDP)-activated deoxy sugars and furthermore enables us to produce novel sorangiosides in a combinatorial biocatalytic approach using three enzyme modules. The SuSy module with the recombinant plant enzyme sucrose synthase (SuSy) and the deoxy sugar module consisting of the enzymes RmlB (4,6-dehydratase), RmlC (3,5-epimerase) and RmlD (4-ketoreductase) from the biosynthetic pathway of dTDP-β-L-rhamnose were combined with the glycosyltransferase module containing the promiscuous recombinant glycosyltransferase SorF from *Sorangium cellulosum* So ce12. Kinetic data and the catalytic efficiency were determined for the donor substrates of SorF: dTDP-α-D-glucose, dTDP-β-L-rhamnose, uridine diphosphate (UDP)-α-D-glucose (Glc), and dTDP-6-deoxy-4-keto-α-D-glucose. The synthesis of glucosyl-sorangioside with *in situ* regeneration of dTDP-Glc was accomplished by combination of SuSy and SorF. The potential of the EMS is demonstrated by combining SuSy, RmlB, RmlC, RmlD with SorF in one-pot for the *in situ* regeneration of dTDP-activated (deoxy) sugars. The HPLC/MS analysis revealed the formation of rhamnosyl-sorangioside and glucosyl-sorangioside, demonstrating the *in situ* regeneration of dTDP-β-L-rhamnose and dTDP-α-D-glucose and a cycle number for dTDP higher than 9. Furthermore, NADH (reduced form of nicotinamdie adenine dinucleotide) regeneration with formate dehydrogenase in the reduction step catalyzed by the 4-ketoreductase RmlD could be integrated in the one-pot synthesis yielding similar conversion rates and cycle numbers. In summary, we have established the first *in situ* regeneration cycle for dTDP-activated (deoxy) sugars by a highly flexible EMS which allows simple exchange of enzymes in the deoxy sugar module and exchange of glycosyltransferases as well as aglycones in the glycosyltransferase module to synthesize new hybrid glycosylated natural products in one-pot.

Keywords: biocatalysis; deoxythymidine 5′-diphosphate (dTDP)-activated sugars; glycosylation; glycosyltransferase; natural products; sorangicin

Introduction

Biological functions of glycoconjugates in animals, plants and microorganisms are mediated by D- and L-deoxyhexoses.[1] A widely used method of nature to change surface properties of compounds and organisms by influencing the interaction with the environment is the presentation of deoxy sugars. The biological function is often drastically altered by a small variation of the involved sugar compound, and recent studies have emphasized the role of deoxy sugars as recognition elements in the action mechanism of drugs.[2] Actinomycetes are the major source of microbial natural products and glycosylation is a very common finding in these secondary metabolites. Another important source of such compounds are the myxobacteria.[3] Interestingly, only a very few glycosylated structures have been reported and no informa-



Scheme 1. Enzyme module system (EMS) for the synthesis of novel glycosylated natural products with *in situ* regeneration of dTDP-activated deoxy sugars.

tion at all is available today about the biochemistry of glycosyl transfer to natural product backbones in myxobacteria. The glycosylation of natural products is catalyzed by glycosyl transferases which requires complex nucleotide sugars as donor substrates. For *in vitro* glycosylation experiments these nucleotide sugars can be synthesized by laborious chemical methods which afford for every single nucleotide sugar its own individual synthesis route with 5 to 20 synthesis steps depending on simple or complex nucleotide sugars, for example, trideoxy sugars, and often results in low yields.[4] An alternative to chemical synthesis is the enzymatic synthesis of nucleotide sugars involving subsequent or one-pot multiple catalytic steps with unprotected sugars in aqueous solution.[5–8] The complex dideoxy sugar dTDP-β-L-olivose and the dideoxy sugar pathway intermediate dTDP-2,6-dideoxy-4-keto-α-D-glucose were thus synthesized starting from 2-deoxyglucose 6-phosphate and dTTP and exploiting the substrate flexibility of the biosynthetic pathway enzymes for dTDP-β-L-rhamnose (dTDP-L-Rha).[6]

Recently, we established an enzyme module system (EMS) for the synthesis of dTDP-activated deoxy sugars starting from dTMP and sucrose (Scheme 1).[7] The SuSy module (**A**) with dTMP-kinase, pyruvate kinase, and sucrose synthase generates the precursor substrate dTDP-α-D-glucose (dTDP-Glc). The deoxy sugar module (**B**) is highly flexible containing biosynthetic deoxy sugar pathways enzymes. dTDP-L-Rha and dTDP-6-deoxy-4-keto-α-D-glucose can now be synthesized in 0.1 and 0.6 g scale (Rupprath et al., unpublished results), respectively, by the combination of both enzyme modules with four enzymes (RmlB, RmlC and RmlD, FDH) in the deoxy sugar module including also NADH cofactor regeneration.

In situ regeneration of NDP-activated sugars during synthesis of glycoconjugates is a versatile approach because isolation procedures of the most often labile NDP-activated sugars and the feedback inhibition of the glycosyltransferase by-product NDP are avoided leading to substantial cost reduction and better reaction performance.[9] Because of these exceptional advances *in situ* regeneration systems for UDP-α-D-glucose (UDP-Glc),[10–14] UDP-α-D-galactose (UDP-Gal),[10–12,15] UDP-α-D-glucuronic acid,[16] CMP-*N*-acetylneuraminic acid,[14,17] GDP-α-D-mannose,[18] GDP-β-L-fucose,[17] UDP-*N*-acteyl-α-D-glucosamine[19] and UDP-*N*-acteyl-α-D-galactosamine[19] nucleotide sugars have been developed. In our previous work we combined SuSy, UDP-Glc 4′-epimerase and β1,4-galactosyltransferase for the production of 0.5 g *N*-acetyllac-

tosamine (LacNAc) in a 3-enzyme *in situ* regeneration system for UDP-Gal.[11,12] We further utilized this regeneration system for UDP-Gal for the synthesis of the Galili-epitope involving β1,4-galactosyltransferase and α1,3-galactosyltransferase.[15,20]

Despite all the benefits of *in situ* regeneration of nucleotide sugars, an *in situ* regeneration system for dTDP-activated sugars has not been established so far. We could recently identify SorF, a glycosyl transferase from the myxobacterium *Sorangium cellulosum* involved in the formation of the antibiotic sorangicin.[21] Applying SorF, we present here the first *in situ* regeneration system for dTDP-activated deoxy sugars by using a generally applicable enzyme module system (EMS, Scheme 1).

Results and Discussion

In the present paper we have extended the EMS for the synthesis of dTDP-deoxy sugars by a third component, the glycosyltransferase module (**C**, Scheme 1). The combination of all three modules in one-pot establishes the first *in situ* regeneration cycle for dTDP-(deoxy) sugars. The cycle is started by catalytic amounts of dTDP and avoids laborious purification procedures for the often labile dTDP-activated deoxy sugars. Also feed-back inhibition of deoxy sugars pathway enzymes[6] in the deoxy sugar module is minimized by coupling it to the glycosyltransferase module. The released dTDP from the glycosyltransferase reaction is subsequently utilized to regenerate dTDP-Glc and the dTDP-deoxy sugar *via* the SuSy and deoxy sugar modules, respectively. We here demonstrate the feasibility of our EMS by utilizing the promiscuous donor substrate acceptance of the glycosyltransferase SorF[21] which leads to the synthesis of novel sorangiosides. In order to optimize the EMS the SorF glycosyltransferase was first kinetically characterized for its donor substrate spectrum.

Kinetic Characterization of the Glycosyltransferase SorF

The SorF glycosyltransferase exhibits substrate flexibility towards dTDP-activated D- and L-sugars.[21] Table 1 summarizes the kinetic values and the catalytic efficiencies of SorF for the donor substrates dTDP-Glc (**1**), dTDP-6-deoxy-4-keto-α-D-glucose (**2**), dTDP-L-Rha (**3**), and UDP-Glc (**4**). The affinity of SorF for **1** was significantly higher (K_M 0.12 mM) than for the proposed natural substrate **4** (K_M 1.69 mM). Although the reaction rate for **4** is better than for **1**, the catalytic efficiency (v_{max}/K_M) for **1** is notably higher suggesting that this is the natural donor substrate of SorF. Interestingly, with **3** SorF shows a reasonable reaction rate and a higher affinity than for **4**.

Table 1. Kinetic data of the donor substrates of the glycosyltransferase SorF.

Substrate	K_M [mM]	v_{max} [U/mg]	v_{max}/K_M
dTDP-α-D-Glc (**1**)	0.12 ± 0.026	1.36 ± 0.026	11.33
dTDP-6-deoxy-4-keto-glucose (**2**)	0.39 ± 0.095	0.48 ± 0.019	1.23
dTDP-β-L-Rha (**3**)	1.01 ± 0.44	0.85 ± 0.1	0.84
UDP-α-D-Glc (**4**)	1.69 ± 0.28	6.78 ± 0.32	4.01

Also the deoxy sugar pathway intermediate **2** is accepted with a similar catalytic efficiency as **3**, but exhibits the lowest reaction rate of all tested donor substrates. The enzyme affinity of SorF for **4** is similar to the described glycosyltransferase GtfB and GtfE (K_M 1.3 mM and 0.72 mM).[22]

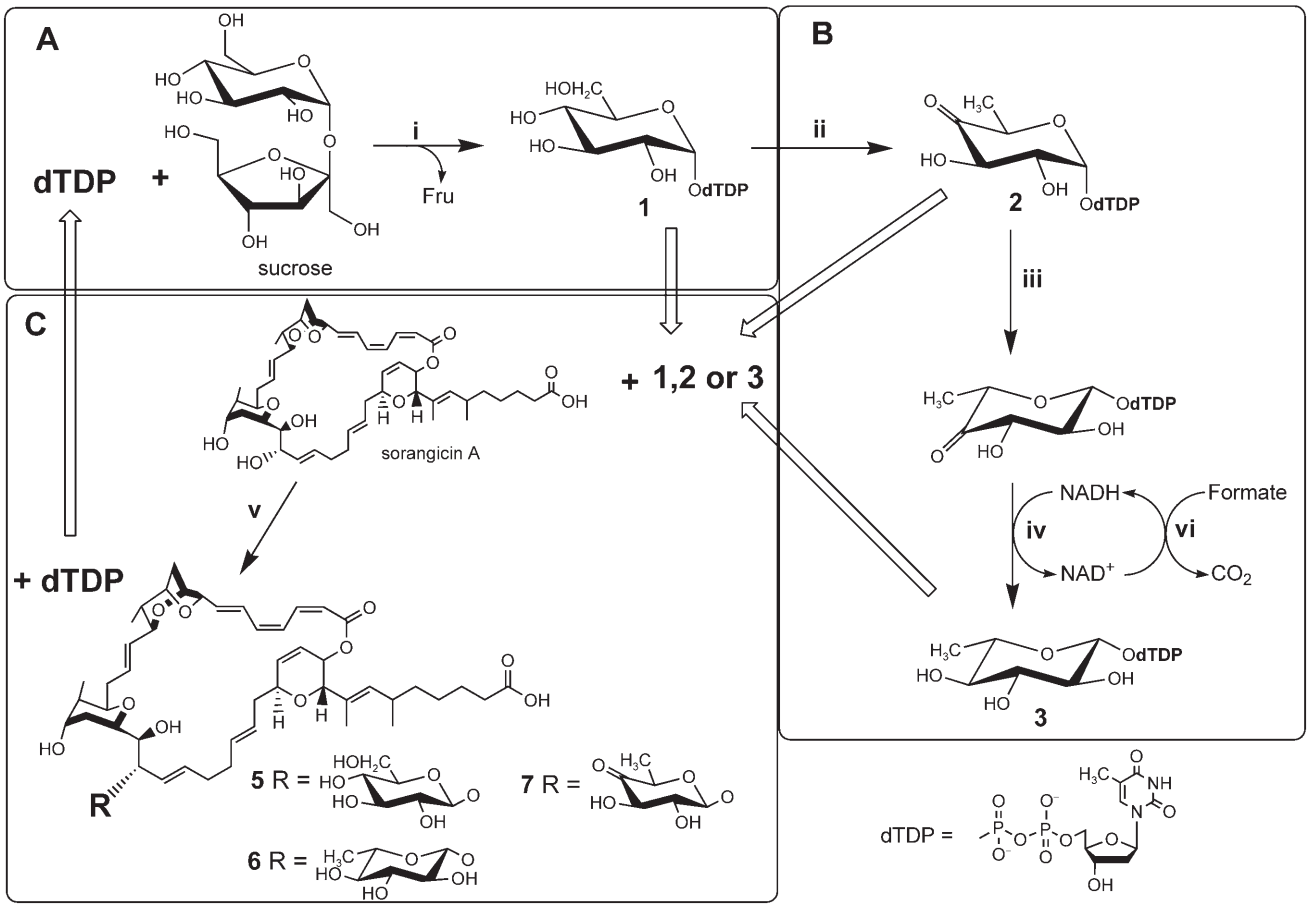
EMS for *in situ* Regeneration of dTDP-Deoxy Sugars

The established EMS for the synthesis of dTDP-deoxy sugars from dTMP and sucrose was extended by the glycosyltransferase module (**C**, Scheme 2). The SuSy module starts with catalytic amounts of dTDP and is combined with the two other modules in an one-pot reaction. Based on the donor substrate flexibility of SorF,[21] two combinations of the modules were tested to demonstrate *in situ* regeneration of the dTDP-activated (deoxy) sugar during synthesis of the corresponding sorangiosides.

The direct combination of the SuSy module and the glycosyltransferase-module (EMS 1 in Scheme 2) led to the formation of **5** with a conversion rate of 97% (Figure 1) for the acceptor substrate sorangicin A as determined by HPLC/MS analysis (Supporting Information). Since only catalytic concentrations of dTDP, namely one-tenth of the acceptor concentration, were used, the maximum cycle number of 10 for dTDP was nearly reached (9.7) demonstrating efficient *in situ* regeneration of **1**.

All three modules were combined to prove *in situ* regeneration of **3** (EMS 2 in Scheme 2). Four different experiments were carried out to optimize the ratios of the enzyme activities. In the first experiment the activities of SuSy and the Rml enzymes were equal. Figure 2 illustrate that **5** is the main product and **6** is only synthesized as a minor product with conversion rates of 59% and 9.2%, respectively (see also Supporting Information). This result reflects the kinetic properties of SorF (Table 1). dTDP-Glc (**1**) has the highest affinity towards SorF and 13-fold greater v_{max}/K_M compared to **3** which favours the formation of the **5**.

At that time optimization of the enzyme activities in the modules came into focus to direct the synthesis



Scheme 2. Enzyme module systems (EMS) for the *in situ* regeneration of dTDP-activated deoxysugars. **EMS 1**: Combination of the SuSy module (**A** with **i** for SuSy) and the glycosyltransferase module (**C** with **v** for SorF) for the synthesis of glucosyl-sorangioside (**5**) with *in situ* regeneration of **1. EMS 2**: Combination of the SuSy module (**A**), the deoxy sugar module (**B** with **ii** for RmlB; **iii** for RmlC; **iv** for RmlD), and the glycosyltransferase module (**C**) for the synthesis of rhamnosylsorangioside (**6**), 6-deoxy-4-keto-glucosylsorangioside (**7**) and **5** with *in situ* regeneration of **3**, **2**, and **1**.

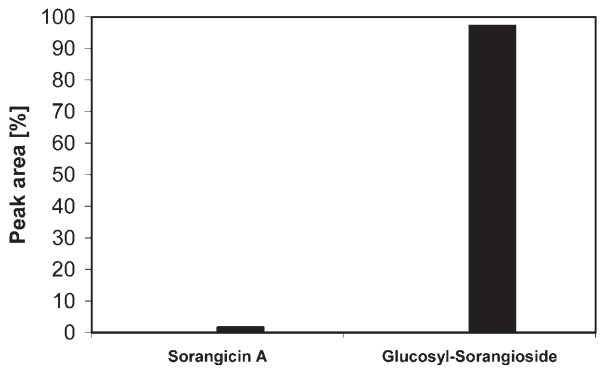


Figure 1. HPLC/MS analysis with peak areas for the educt sorangicin A and the product glucosyl-sorangioside **5** in enzyme module system 1 (EMS 1, Scheme 2). The SuSy module (**A**) and the glycosyltransferase module (**C**) with SorF were combined for *in situ* regeneration of **1**. The peak area of product **5** corresponds to the conversion rate of the educt.

of the favoured glycosylated compound (Figure 2 and Supporting Information). In the second experiment the activities of RmlB (5-fold), RmlC (2.4-fold) and RmlD (2.4-fold) in the deoxy sugar module were increased to prevent the accumulation of **1** and to force the formation of **3** as substrate for the transfer reaction. These conditions resulted in the formation of **6** as the main product with a conversion rate of 57%. However, **5** and **7** were also detected by HPLC/MS with conversion rates of 32% and 6%, respectively (Figure 2). The cycle number for dTDP reached 9.5 (maximum 10) demonstrating again efficient *in situ* regeneration of the dTDP-(deoxy) sugars. Most importantly, dTDP was cycled nearly 6 times throughout the whole EMS with five enzymes involved in the regeneration of **3**. However, the conversion of dTDP-Glc led still to the formation of **5**, because of the high affinity and high reaction rate of SorF with **1** (Table 1).

In the third experiment the activity ratio of RmlB/SorF was further increased to give high excess of

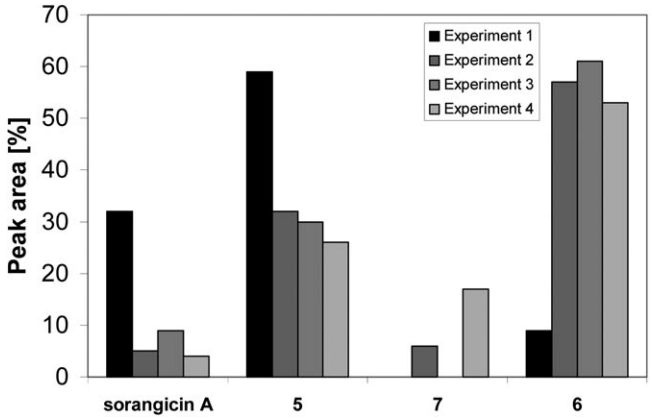


Figure 2. HPLC/MS analysis with peak areas for the educt sorangicin A and the products glucosyl-sorangioside **5**, rhamnosyl-sorangioside **6**, and 6-deoxy-4-keto-glucosyl-sorangioside **7** in enzyme module system 2 (EMS 2, Scheme 2). The three modules **A**, **B** and **C** in Scheme 2 were combined for *in situ* regeneration of the donor substrates **1**, **2**, and **3**. Enzyme ratios in the enzyme modules were varied in experiments 1 to 4 as described in the Experimental Section. The peak areas of the products correspond to the conversion rate of the educt.

RmlB and to force the synthesis of **3**. Figure 2 illustrate that only **6** and **5** with conversion rates of 61% and 30%, respectively, were synthesized (see also Supporting Information). dTDP is recycled more than 6 times in the EMS running through all enzymatic steps for the *in situ* regeneration of **3**. All together more than 9 cycles of dTDP were achieved for the *in situ* regeneration of **1** and **3** as donor substrates of SorF (maximum cycle number is 10). Most interestingly, compound **7** was not formed under these conditions, which is probably a result of the reduced SorF activity.

Finally, to demonstrate that *in situ* regeneration of NADH (Scheme 2) can also be integrated into the EMS, formate dehydrogenase (FDH) was added together with the substrate sodium formate to generate NADH from NAD^+ as cofactor for the reaction of RmlD (experiment 4 in Figure 2 and Supporting Information). HPLC/MS analysis clearly demonstrates that the EMS with 6 enzymes generates **6** as the main product with a conversion rate of 53%. Together with the products **5** (26%) and **7** (17%) a conversion rate of 96% is reached for the acceptor sorangicin A. Again dTDP reaches almost the maximum cycle number of 10 including 5 cycles for the *in situ* regeneration of **3.** Thus, NAD^+ also reaches a cycle number of 5 (maximum 10).

Besides the improvement of product formation demonstrated by these four experiments, further optimization of the *in situ* regeneration of dTDP-activated deoxy sugars could be facilitated by the utilization of genetic algorithms.[23]

Recently, Eguchi et al. and Thorson et al. reported on the *in situ* generation of complex dTDP-deoxy sugars by the reverse reaction of glycosyltransferases involved in the formation of vicenistatin, calichamycin, and vancomycin.[25] The reactions resemble on first sight those of sucrose synthase (SuSy) which we used to generate dTDP-Glc by the reverse reaction of this plant glycosyltransferase. However, sucrose is a high energy substrate and the synthesis of NDP-Glc with SuSy can be readily optimized towards high enzyme productivities as demonstrated by our previous work.[7,8,12,26] In the synthesis of **1** and **3** from sucrose we reach for the SuSy reaction turnover numbers of 33,000 and 440 (μmol product/μmol enzyme), respectively.[7] In contrast, the formation of dTDP-deoxy sugars from calichamycins or vancomycins by the reverse reaction of glycosyltransferase reaches only turnover numbers between 5 and 10, which is due to relatively high enzyme concentrations in the μM range.[25b] Moreover, for the enzymatic aglycone switch using the reverse reaction of the glycosyltransferase VinC, Eguchi et al. report a maximum conversion yield of 42% for the acceptor substrate with a 3-fold molar excess of the enzyme which corresponds to a turnover number of 0.14.[25a]

Conclusions

In summary, we have demonstrated that dTDP-activated (deoxy) sugars like **1**, **2**, and **3** are regenerated *in situ* using an enzyme module system consisting of up to 6 enzymes. Other systems where the NDP-sugar is generated by a pyrophosphorylase, for example, RmlA,[24] may also be used, but are difficult to optimize due to the larger number of enzymes needed for regeneration of the nucleotide sugar (eight enzymes starting from D-glucose) and the sensitive feedback inhibition of RmlA (22 μM)[6] by **3**. In our EMS we could also demonstrate that the use of a promiscuous glycosyltransferase yielded differently glycosylated products. Extension of our EMS by other deoxy sugar pathway enzymes should also yield libraries of glycosylated natural compounds. On first sight, the combination of many enzymes seems to be a disadvantage. However, many dTDP-deoxy sugar pathways have been already set up *in vitro*.[8,27]

Experimental Section

dTDP-6-deoxy-4-keto-α-D-glucose (**2**) and dTDP-L-Rha (**3**) were synthesized as described elsewhere.[7] dTDP, **1** and **4** were purchased from Sigma–Aldrich. Formate dehydrogenase was from Julich Chiral Solutions (Jülich, Germany). NADH, Tris buffer, $MgCl_2$, ammonium formate, BSA and IPTG were purchased from Roth (Karlsruhe, Germany);

yeast extract and tryptone peptone were from BD Biosciences (Heidelberg, Germany). Sorangicin A was provided by Dr. Irschik (Helmholtz Centre for Infection Research, Braunschweig, Germany) as described before.[21]

Production and Purification of SorF

The production and purification of SorF was carried out as described elsewhere.[21] The fermentation was scaled up to 10-L scale. An ED10 B. Braun (Melsungen, Germany) fermenter was filled with 10 L LB medium and inoculated with a 200 mL LB preparatory culture, which was incubated for 18 h at 37 °C in an HT shaker (Infors, Einsbach, Germany). The *E. coli* cells were grown at 37 °C until an $OD_{600} = 0.5$–0.7 was reached. The oxygen partial pressure was regulated dynamically with the stirrer speed using a threshold concentration of 40 % with an air flow of 10 L/min. Production of SorF was induced with 0.4 mM IPTG, followed by incubation for 24 h at 16 °C. The cells were then harvested by centrifugation using a RC5B centrifuge (Thermo Electron, Langenselbold, Germany).

Production and Purification of RmlB, RmlC, RmlD, and SuSy

The cloning, expression and purification were performed as described and published elsewhere.[6,7,28]

Kinetic Data of SorF

The maximum reaction rate v_{max} and the Michaelis constant K_M were determined for the donor substrates dTDP-Glc (**1**), dTDP-6-deoxy-4-keto-α-D-glucose (**2**), dTDP-L-Rha (**3**), and UDP-Glc (**4**). In a total volume of 20 μL the Tris-HCl buffer (50 mM), $MgCl_2$ (10 mM), sorangicin A (1.4 mM), BSA (1 mg mL^{-1}), SorF (20 mU mL^{-1} and 10 mU mL^{-1}) were mixed with the NDP-activated sugars (0.5, 1, 2, 4, 6, 8 and 10 mM). After starting the reaction with sorangicin A, the samples were incubated for 5 min at 30 °C and then stopped by heating (95 °C) for 30 s. The samples were centrifuged for 15 min at 15000 rpm (Rotina 35R, Hettich, Germany) and subsequently analyzed by CE (see below). Specific enzyme activities were determined from the produced dTDP, and the corresponding protein concentrations.[29] The v_{max} and K_M values were calculated by non-linear regression using the Michaelis–Menten-equation (Sigma Plot, Systat Software GmbH, Erkrath, Germany).

Enzyme Module System 1 (EMS 1)

The SuSy and the glycosyltransferase modules (Scheme 2) containing purified SuSy and SorF were combined. In a total volume of 500 μL Tris-HCl buffer (50 mM, pH 7.5), $MgCl_2$ (10 mM), sucrose (250 mM), sorangicin A (1.4 mM), SuSy (1 U mL^{-1}), and SorF (0.13 U mL^{-1}) were mixed. The reaction was started by adding dTDP (0.14 mM). The samples were incubated at 30 °C for 18 h and then stopped by heating (95 °C) for 3 min. Subsequently, the formed sorangiosides were purified by solid-phase extraction and analysed by HPLC/MS (see below).

Enzyme Module System 2 (EMS 2)

The three modules (Scheme 2) containing the purified enzymes SuSy, RmlB (4,6-dehydratase), RmlC (3,5-epimerase), RmlD (4-ketoreductase), and SorF were combined. Four experiments were performed as described below for optimization of product formation and demonstration of NADH *in situ* regeneration using formate dehydrogenase. All samples were incubated and worked up as described above.

Experiment 1: In a total volume of 500 μL Tris-HCl buffer (50 mM, pH 7.5), $MgCl_2$ (10 mM), sucrose (250 mM), sorangicin A (1.4 mM), SuSy (1 U mL^{-1}), RmlB (1 U mL^{-1}), RmlC (1 U mL^{-1}), RmlD (1 U mL^{-1}), NADH (1.4 mM), and SorF (0.13 U mL^{-1}) were mixed. The reaction was started by adding the dTDP (0.14 mM).

Experiment 2: The conditions in experiment 1 were modified by increasing the enzyme activities in module B to RmlB (5 U mL^{-1}), RmlC (2.4 U mL^{-1}), and RmlD (2.4 U mL^{-1}). All other parameters remained as described for experiment 1.

Experiment 3: The SorF activity was reduced to 0.065 U mL^{-1} and the RmlB activity increased to 10 U mL^{-1}. RmlC and D activities and conditions remained as described in experiment 2.

Experiment 4: NADH *in situ* regeneration by formate dehydrogenase should be demonstrated in the EMS. In a total volume of 500 μL Tris-HCl buffer (50 mM, pH 7.5) $MgCl_2$ (10 mM), sucrose (250 mM), sorangicin A (1.4 mM), SuSy (1 U mL^{-1}), RmlB (5 U mL^{-1}), RmlC (1.2 U mL^{-1}), RmlD (1.2 U mL^{-1}), NAD^+ (0.14 mM), FDH (5 U mL^{-1}), ammonium formate (200 mM), and SorF (0.13 U mL^{-1}) were mixed. The reaction was started by adding dTDP (0.14 mM).

Analysis of Sorangicin and the Sorangiosides

After stopping the EMS by heating, samples were centrifuged for 15 min at 15000 rpm (Rotina 35R, Hettich, Tuttlingen, Germany) and products purified by solid-phase extraction on Sep-Pak C18 columns (Waters, Eschborn, Germany) using methanol. The samples were evaporated in a speed-vac, and the remaining residue was dissolved in 200 μL methanol for HPLC/MS analysis. A DAD-HPLC (Agilent 1100 Serie) coupled to an electrospray ionization device connected to an ion trap of an HCT plus MS was employed (Bruker, Bremen, Germany). HPLC was carried out on a reversed phase Nucleodur C18 column 125 × 2 mm/3 μm (Macherey–Nagel, Düren, Germany) using different gradients ranging from 95 % solvent A (water + 0.1 % formic acid) to 95 % solvent B (acetonitrile + 0.1 % formic acid) with a flow rate of 0.4 mL/min.

Capillary Electrophoresis of NDP and NDP-Sugars

The samples for the determination of kinetic data (v_{max} and K_M) of the NDP-activated donor substrates of SorF were stopped by heating (95 °C) for 0.5 min, centrifuged for 15 min at 15000 rpm (Rotina 35R, Hettich, Tuttlingen, Germany) and examined by analysis of the side products. The formation of dTDP and UDP was monitored by capillary electrophoresis on a P/ACE MDQ apparatus from Beckman Coulter (Krefeld, Germany), equipped with a UV detector. Separation of NDP and NDP-sugars was accomplished on

an untreated fused-silica capillary (I.D. 75 μm, 57 cm total capillary length, 50 cm to the detector) with 50 mM $Na_2B_4O_7{\cdot}10H_2O$/64 mM boric acid buffer, pH 8.9. Conditions for migration and detection were 25 kV (23 μA) at 25 °C and UV detection at 254 nm, respectively. Samples were injected by pressure (5.0 sec at 0.5 psi in the forward direction). The conversion of NDP-sugars by SorF was calculated by determination of NDP concentrations using standard calibration curves for dTDP and UDP, respectively.

Acknowledgements

L.E. and C.R. dedicate this paper to Prof. Dr. Dr. h.c. Maria-Regina Kula on the occasion of her 70th birthday. L.E. thanks Prof. Kula for her constant support and fruitful discussions during research at the former Institute of Enzyme Technology of the Heinrich-Heine-University Düsseldorf at the Research Centre Jülich, Germany. Financial support through the EU grant "GENOVA" (Contract No. QLK3–999–00095) to L.E. is gratefully acknowledged. The authors thank Daniel Krug for help with HPLC-MS analysis of sorangicin and its derivatives. Research in the laboratory of R.M. was supported by the Deutsche Forschungsgemeinschaft (DFG) and the Bundesministerium für Bildung und Forschung (BMB+F).

References

[1] a) S. Hanessian, *Adv. Carbohydr. Chem.* **1966**, *21*, 143–207; b) T. Reichstein, E. Weiss, *Adv. Carbohydr. Chem.* **1962**, *17*, 65–120; c) N. Williams, J. Wander, in: *The Carbohydrates, Chemistry and Biochemistry*, Vol. 1B, (Eds.: W. Pigman, D. Horton), Academic Press, New York, **1980**, pp 761–798; d) J. F. Kennedy, C. A. White, *Bioactive carbohydrates in chemistry, biochemistry and biology*, Ellis Horwood Publishers, Chichester, **1983**.

[2] a) S. M. Poulsen, C. Kofoed, B. Vester, *J. Mol. Biol.* **2000**, *304*, 471–481; b) J. R. Menninger, *J. Basic Clin. Physiol. Pharmacol.* **1995**, *6*, 229–250; c) F. Schlunzen, R. Zarivach, J. Harms, A. Bashan, A. Tocilj, R. Albrecht, A. Yonath, F. Franceschi, *Nature* **2001**, *413*, 814–821; d) J. S. Thorson, T. J. J. Hosted Jr., J. Jiang, B. J. B. , J. Ahlert, *Curr. Org. Chem.* **2001**, *5*, 139–167; e) J. L. Hansen, J. A. Ippolito, N. Ban, P. Nissen, P. B. Moore, T. A. Steitz, *Mol. Cell* **2002**, *10*, 117–128; f) F. Schlunzen, J. M. Harms, F. Franceschi, H. A. S. Hansen, H. Bartels, R. Zarivach, A. Yonath, *Structure* **2003**, *11*, 329–338; g) M. Gaynor, A. S. Mankin, *Curr. Top. Med. Chem.* **2003**, *3*, 949–960.

[3] K. Gerth, S. Pradella, O. Perlova, S. Beyer, R. Müller, *J. Biotechnol.* **2003**, *106*, 233–253.

[4] a) J. G. Moffat, in: *Methods in Enzymology*, Vol. 8, (Ed.: F. Ginsburg), Academic Press, New York, London, **1966**, pp 136–142; b) V. Wittmann, C. H. Wong, *J. Org. Chem.* **1997**, *62*, 2144–2147; c) F. Cramer, H. Neuenhoeffer, K. H. Scheit, G. Schneider, J. Tennigkeit, *Angew. Chem.* **1962**, *74*, 387; d) F. Cramer, H. Schaller, H. A. Staab, *Chem. Ber.* **1961**, *94*, 1612–1621; e) M. Arlt, O. Hindsgaul, *J. Org. Chem.* **1995**, *60*, 14–15; f) G. Baisch, R. Oehrlein, *Bioorg Med Chem.* **1997**, *5*, 383–391.

[5] T. Bülter, L. Elling, *Glycoconjugate J.* **1999**, *16*, 147–159.

[6] S. Amann, G. Dräger, C. Rupprath, A. Kirschning, L. Elling, *Carbohydr. Res.* **2001**, *335*, 23–32.

[7] L. Elling, C. Rupprath, N. Günther, U. Römer, S. Verseck, P. Weingarten, G. Dräger, A. Kirschning, W. Piepersberg, *ChemBioChem.* **2005**, *6*, 1423–1430.

[8] C. Rupprath, T. Schumacher, L. Elling, *Curr. Med. Chem.* **2005**, *12*, 1637–1675.

[9] a) L. Elling, in: *Advances in Biochemical Engineering/Biotechnology*, Vol. 58, (Ed.: T. Scheper), Springer-Verlag, Berlin, **1997**, pp 89–144; b) K. M. Koeller, C. H. Wong, *Chem. Rev.* **2000**, *100*, 4465–4493.

[10] a) C. H. Wong, S. L. Haynie, G. M. Whitesides, *J. Org. Chem.* **1982**, *47*, 5416–5418; b) T. Noguchi, T. Shiba, *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1594–1596.

[11] L. Elling, M. Grothus, M.-R. Kula, *Glycobiology* **1993**, *3*, 349–355.

[12] A. Zervosen, L. Elling, *J. Am. Chem. Soc.* **1996**, *118*, 1836–1840.

[13] a) M. Ichikawa, R. L. Schnaar, Y. Ichikawa, *Tetrahedron Lett.* **1995**, *36*, 8731–8732; b) Y. Ichikawa, R. Wang, C. H. Wong, *Meth. Enzymol.* **1994**, *247* 107.

[14] Y. Ichikawa, J. L.-C. Liu, G.-J. Shen, C. H. Wong, *J. Am. Chem. Soc.* **1991**, *113*, 6300–6302.

[15] C. H. Hokke, A. Zervosen, L. Elling, D. H. Joziasse, D. H. van den Eijnden, *Glycoconj. J.* **1996**, *13*, 687–692.

[16] D. Gygax, P. Spies, T. Winkler, U. Pfaar, *Tetrahedron* **1991**, *47*, 5119–5122.

[17] Y. Ichikawa, Y. C. Lin, D. P. Dumas, G. J. Shen, E. Garciajunceda, M. A. Williams, R. Bayer, C. Ketcham, L. E. Walker, J. C. Paulson, C. H. Wong, *J. Am. Chem. Soc.* **1992**, *114*, 9283–9298.

[18] P. Wang, G. J. Shen, Y. F. Wang, Y. Ichikawa, C. H. Wong, *J. Org. Chem.* **1993**, *58*, 3985–3990.

[19] G. C. Look, Y. Ichikawa, G. J. Shen, P. W. Cheng, C. H. Wong, *J. Org. Chem.* **1993**, *58*, 4326–4330.

[20] N. Brinkmann, M. Malissard, M. Ramuz, U. Römer, T. Schumacher, E. G. Berger, L. Elling, C. Wandrey, A. Liese, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2503–2506.

[21] M. Kopp, C. Rupprath, H. Irschik, A. Bechthold, L. Elling, R. Müller, *ChemBioChem.* **2007**, *8*, 813–819.

[22] A. M. Mulichak, H. C. Losey, C. T. Walsh, R. M. Garavito, *Structure (Camb)* **2001**, *9*, 547–557.

[23] C. Hoh, G. Dudziak, A. Liese, *Bioorg. Med. Chem. Lett.* **2002**, 1031–1034.

[24] Y. B. Kang, Y. H. Yang, K. W. Lee, S. G. Lee, J. K. Sohng, H. C. Lee, K. Liou, B. G. Kim, *Biotechnol. Bioeng.* **2006**, *93*, 21–27.

[25] a) A. Minami, K. Kakinuma, T. Eguchi, *Tetrahedron Lett.* **2005**, *46*, 6187–6190; b) C. S. Zhang, B. R. Griffith, Q. Fu, C. Albermann, X. Fu, I. K. Lee, L. J. Li, J. S. Thorson, *Science* **2006**, *313*, 1291–1294; c) H. B. Bode, R. Müller, *Angew. Chem.* **2007**, *119*, 2195–2198.

[26] a) A. Zervosen, A. Stein, H. Adrian, L. Elling, *Tetrahedron* **1996**, *52*, 2395–2404; b) T. Bülter, C. Wandrey, L. Elling, *Carbohydr. Res.* **1997**, *305*, 469–473; c) A. Stein, M. R. Kula, L. Elling, *Glycoconjugate J.* **1998**, *15*, 139–145; d) U. Römer, N. Nettelstroth, W. Köckenberger, L. Elling, *Adv. Synth. Catal.* **2001**, *343*, 655–661.

[27] a) T. M. Hallis, H. W. Liu, *Acc. Chem. Res.* **1999**, *32*, 579–588; b) A. Trefzer, J. A. Salas, A. Bechthold, *Nat. Prod. Rep.* **1999**, *16*, 283–299.
[28] U. Römer, H. Schrader, N. Günther, N. Nettelstroth, W. B. Frommer, L. Elling, *J. Biotechnol.* **2004**, *107*, 135–149.
[29] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–254.

