Vancomycin–Oligosaccharide Library

A Programmable One-Pot Oligosaccharide Synthesis for Diversifying the Sugar Domains of Natural Products: A Case Study of Vancomycin

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Drug discovery relies heavily on the pool of natural products for the identification of lead compounds. Numerous natural products are glycosylated, and their peripheral carbohydrate moieties are the key recognition elements and often define their biological activities.[1] Yet, efficient methods to systematically alter these essential carbohydrate ligands are still lacking. There are several strategies for the one-pot synthesis of oligosaccharides.[2] In particular, programmed one-pot oligosaccharide synthesis facilitates the convenient assembly of glycan structures[3] and has been used in the synthesis of such complex molecules as Globo H,[4] Lewis Y,[5] and fucosyl GM_1.[6] Desired glycan structures three to six residues in length can be constructed by choosing building blocks with suitable reactivities from the Optimer database.[7] Furthermore, the one-pot glycosylation methodology may provide a unique opportunity for convenient assembly of glycosylation libraries of natural products (Figure 1). Libraries of this type have been shown to facilitate elucidation of glycan function as well as the discovery of new compounds with interesting activities.[8]

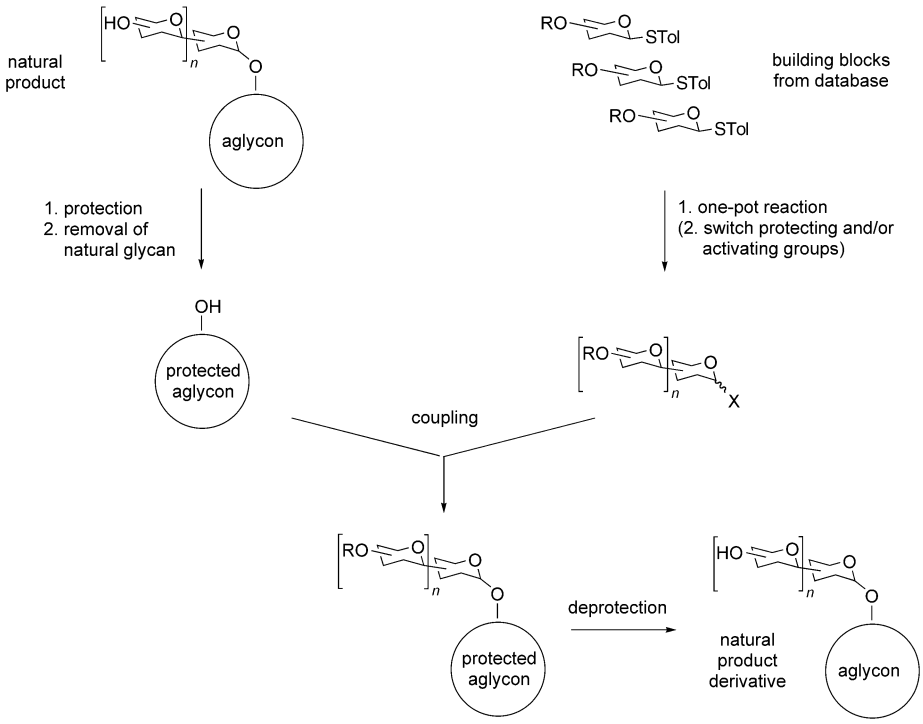

Figure 1. Strategies for the synthesis of libraries of glycosylated natural products by the direct one-pot glycosylation (left) or the coupling of preformed oligosaccharides (right).

The notoriously difficult glycosylation of vancomycin as well as the clinical importance of this antibiotic prompted us to choose vancomycin as a test case for the strategy outlined in Figure 1. Vancomycin is the most important member of the glycopeptide class of antibiotics and is considered the drug of last resort for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Due to the emergence of vancomycin-intermediate-resistant *S. aureus* (VISA) and vancomycin-resistant enterococci (VRE), there is currently great interest in improving the efficacy of vancomycin. Vancomycin inhibits peptidoglycan biosynthesis in the bacterial cell wall by binding to the terminal peptide fragments of immature cell walls. In VRE a change in the peptide substrate weakens its affinity for the vancomycin binding pocket. Although the hydrogen-bonding interactions between the vancomycin aglycon and the peptidoglycan precursor are well understood, reengineering of the binding pocket by replacing internal amino acids would be a very complex synthetic endeavor. However, dramatic increases in activity against resistant strains were achieved by attaching hydrophobic groups to vancomycin's terminal sugar residue, vancosamine.[9] This increased activity was originally rationalized by the enhancing effects the substituents might have on dimerization and membrane anchoring.[10] More detailed studies suggest that the new vancomycin derivatives inhibit peptidoglycan biosynthesis by directly interfering with the enzymes catalyzing transglycosylation.[11–13]

In order to further investigate the role of the glycan portion, an efficient strategy for the glycosylation of the vancomycin aglycon is required. Enzymatic glycosylation procedures are restricted by the substrate tolerance of the glycosyltransferases and the availability of the nucleotide-sugar donors.[14–16] Previous chemical protocols suffer from poor yields in the glycosylation step[17] or lengthy protection/deprotection procedures.[18] A solid-support approach was reported, greatly simplifying workup and purification procedures.[19] However, only monoglycosylated derivatives resulted from this work. In a different approach, a short ethylene glycol linker was placed between the glycan and the aglycon, simplifying the coupling step significantly.[20] Yet, no

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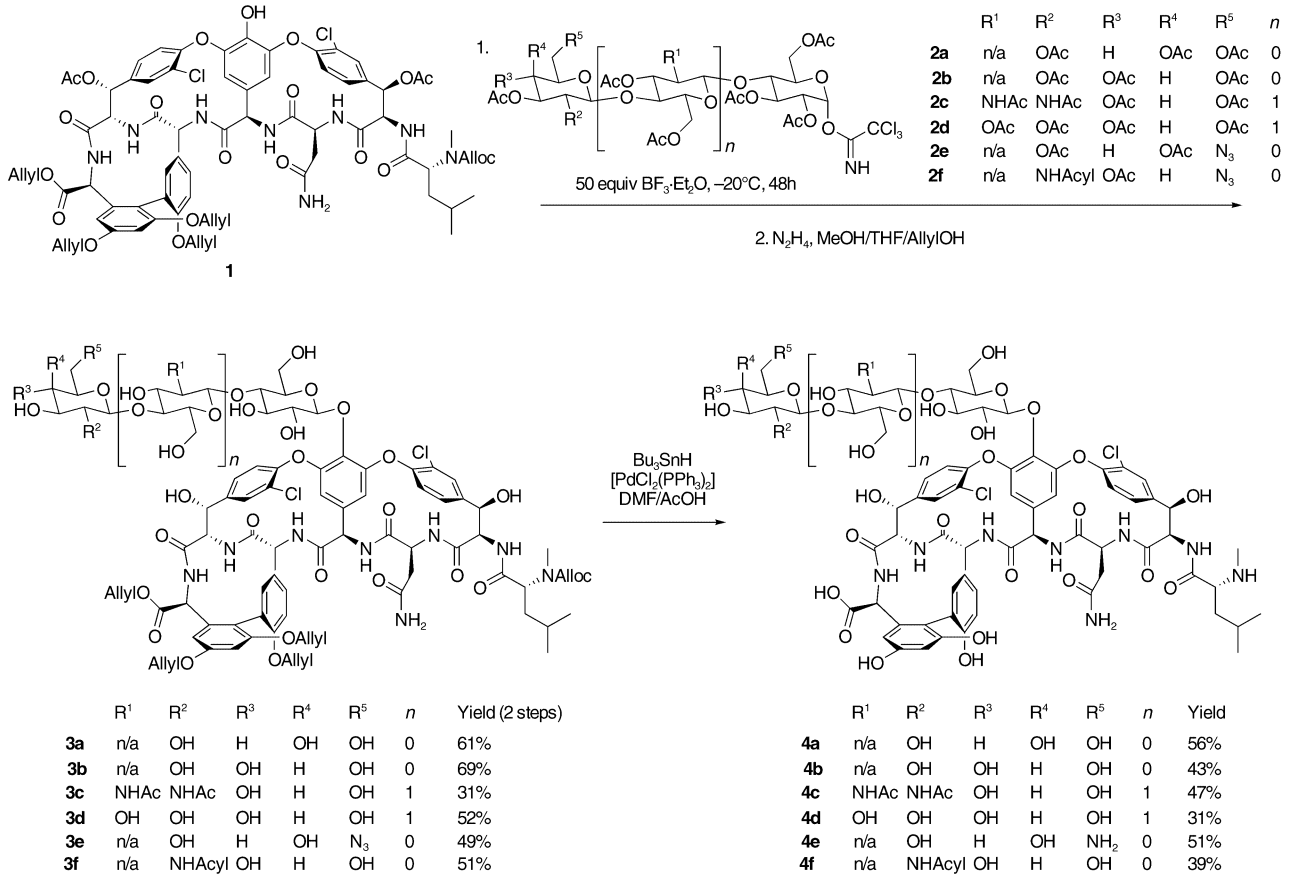
examples of direct chemical glycosylation of the aglycon to provide vancomycin derivatives bearing nonnatural di- or oligosaccharide substituents have been reported. By implementing a programmable one-pot oligosaccharide strategy, we hoped to construct a set of such derivatives.

In order to investigate the feasibility of our approach, it was necessary to establish an efficient glycosylation procedure for attaching oligosaccharides to a protected vancomycin aglycon. Di-*O*-acetyl-*N*-alloc-tri-*O*-allyl vancomycin aglycon allyl ester **1** was obtained in seven steps from commercially available vancomycin.[17] Glycosylation of **1** with thioglycoside donors could not be achieved under a variety of conditions, including different promoters such as *N*-iodosuccinimide and dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST). Therefore, glycosylation with peracetylated imidate donors **2a** and **2b** was performed and proceeded in good yields after optimization (Scheme 1). The acetate and allyl protecting groups were subsequently removed by reaction with hydrazine and palladium-catalyzed reduction, respectively,[17] providing the vancomycin derivatives **4a** and **4b**.[21]

The successful synthesis of oligosaccharides by the one-pot strategy relies on the generation of significant differences in reactivity between the individual thioglycoside donors. New nitrogen-containing building blocks were designed by fine-tuning reactivities through various electron-donating and -withdrawing protecting groups (Figure 2). Relative reactivity values (RRV) of these compounds were determined as described previously.[7] As shown in Scheme 2, the reactivity differences of the new donors and building blocks available from our database are in a suitable range for successful one-pot syntheses. The oligosaccharides were assembled according to the standard coupling protocol of the programmable one-pot synthesis (*N*-iodosuccinimide, triflic acid). Following the synthesis of the oligosaccharides, the protecting groups were exchanged for acetates. This operation minimizes the number of deprotection steps required after coupling of the glycans to the vancomycin aglycon. The peracetylated oligosaccharides were activated as trichloroacetimidates **2c–f** by a two-step procedure: deprotection of the anomeric hydroxy groups (hydrazine/acetate in the case of anomeric acetate, *N*-bromosuccinimide in the case of anomeric thioglycosides) followed by conversion to their corresponding trichloroacetimidates with trichloroacetonitrile and cesium carbonate. Compounds **2c–f** were then coupled to the vancomycin aglycon by using the conditions developed for the couplings of **2a** and **b**, and the protecting groups were removed. Deallylation of **3e** occurred with concomitant reduction of the C6 azide on the terminal sugar residue by means of a radical-mediated reduction mechanism through a Sn–N bond-forming process.

The biological activities of the vancomycin derivatives were investigated by determination of their minimum inhibitory concentrations (MIC) against a vancomycin-sensitive



	R¹	R²	R³	R⁴	R⁵	n
2a	n/a	OAc	H	OAc	OAc	0
2b	n/a	OAc	OAc	H	OAc	0
2c	NHAc	NHAc	OAc	H	OAc	1
2d	OAc	OAc	OAc	H	OAc	1
2e	n/a	OAc	H	OAc	N_3	0
2f	n/a	NHAcyl	OAc	H	N_3	0

	R¹	R²	R³	R⁴	R⁵	n	Yield (2 steps)
3a	n/a	OH	H	OH	OH	0	61%
3b	n/a	OH	OH	H	OH	0	69%
3c	NHAc	NHAc	OH	H	OH	1	31%
3d	OH	OH	OH	H	OH	1	52%
3e	n/a	OH	H	OH	N_3	0	49%
3f	n/a	NHAcyl	OH	H	OH	0	51%

	R¹	R²	R³	R⁴	R⁵	n	Yield
4a	n/a	OH	H	OH	OH	0	56%
4b	n/a	OH	OH	H	OH	0	43%
4c	NHAc	NHAc	OH	H	OH	1	47%
4d	OH	OH	OH	H	OH	1	31%
4e	n/a	OH	H	OH	NH_2	0	51%
4f	n/a	NHAcyl	OH	H	OH	0	39%

Scheme 1. Coupling of various oligosaccharides to vancomycin aglycon and deprotection of the resulting vancomycin derivatives. n/a = not available. Alloc = allyloxycarbonyl.

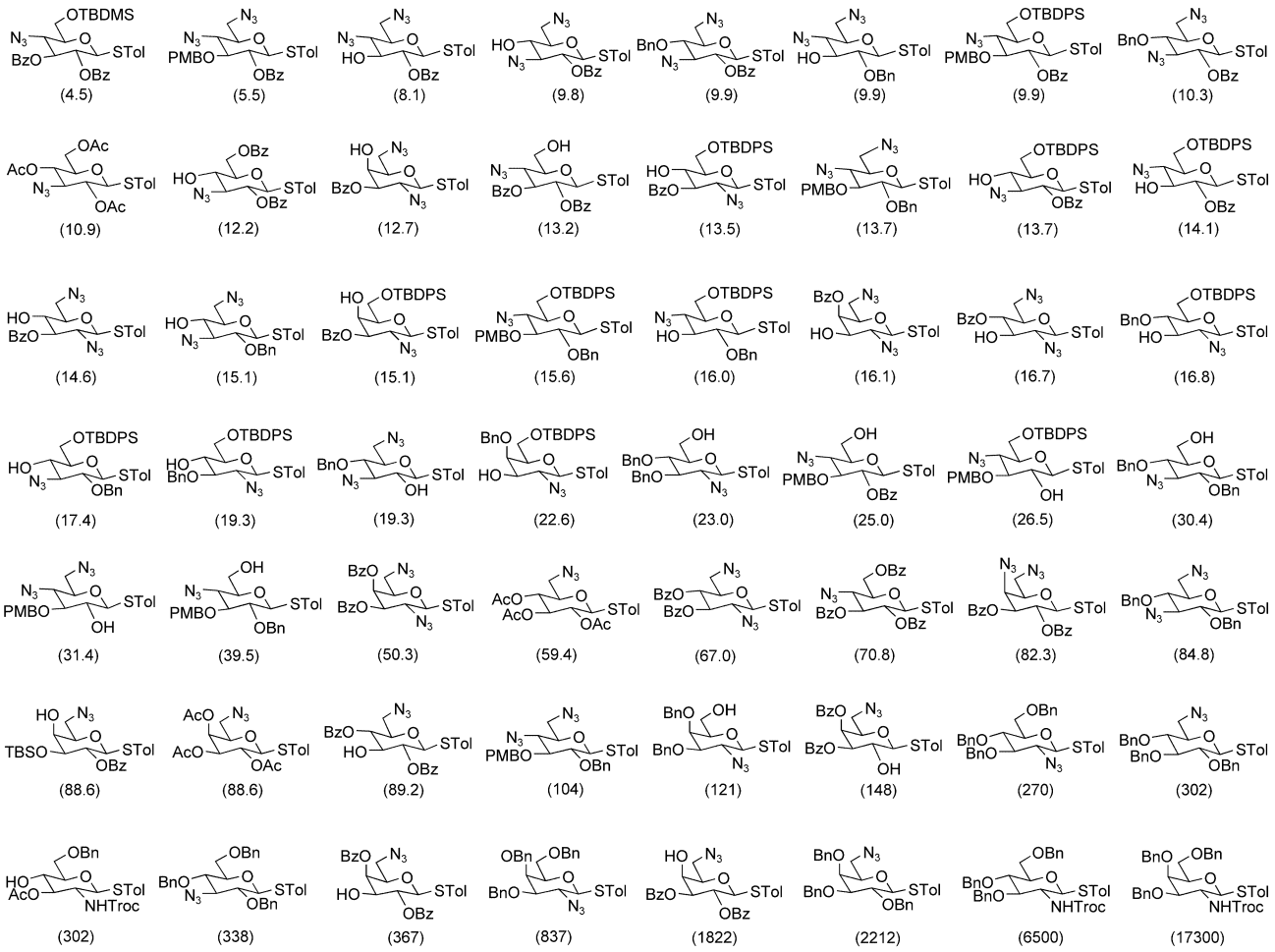


Figure 2. New nitrogen-containing building blocks for the Optimer database. Relative reactivity values are in parentheses. Bn = Benzyl, PMB = *para*-methoxybenzyl, TBDMS = *tert*-butyldimethylsilyl, TBDPS = *tert*-butyldiphenylsilyl, Tol = toluene.

(*E. faecalis* 29212, MIC(vancomycin) = 2 μg mL^{-1}) and a Van A vancomycin-resistant strain (*E. faecalis* BM4166, MIC(vancomycin) = 2000 μg mL^{-1}). Compounds **4e–f** were shown to inhibit growth of vancomycin-sensitive bacteria at a concentration of 5 μg mL^{-1}, whereas compounds **4a–d** showed no growth inhibition at concentrations up to 40 μg mL^{-1}. This result and other data (not shown) indicate the importance of the amino functionality on the terminal sugar for antibacterial activity. Growth inhibition of the Van A strain by **4a–f** was not observed at concentrations up to 100 μg mL^{-1}.

In conclusion, by combining an efficient oligosaccharide segment glycosylation with a straightforward deprotection scheme, we have developed a method for the facile synthesis of vancomycin derivatives bearing different sugar substituents. Compounds **4a–f** are the first products of the one-step chemical glycosylation of the vancomycin aglycon with non-natural oligosaccharides. Their biological evaluation demonstrates the importance of the glycan portion for activity, in particular the requirement for a terminal amino sugar residue. The chemistry developed sets the stage for a more detailed, systematic exploration of glycan function. Furthermore, this study constitutes the first implementation of the programmable one-pot glycosylation towards the synthesis of a set of natural product derivatives. This new strategy, that is, rapid creation of oligosaccharide sets by one-pot glycosylation followed by conversion to peracetylated trichloroacetimidate donors and coupling to an aglycon, should be generally applicable towards the construction of glycosylation libraries of natural products and the exploration of the varied biological functions of their carbohydrate substituents.

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Keywords: antibiotics · carbohydrates · glycosylation · oligosaccharides · vancomycin

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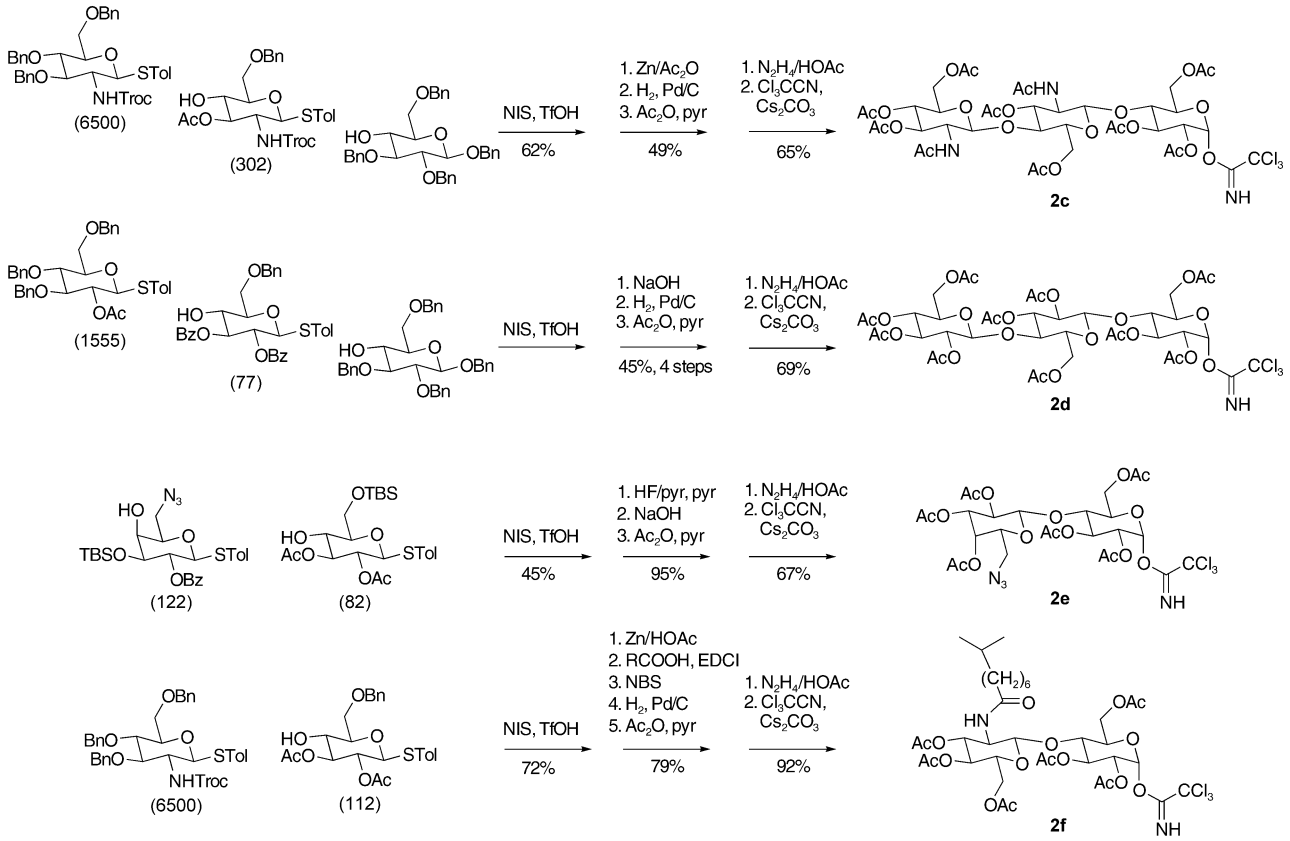

Scheme 2. Programmable one-pot oligosaccharide synthesis, followed by switching of protecting groups and activation as trichloroacetimidates. EDCI = 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride, pyr = pyridine, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl, Troc = 2,2,2-trichloroethoxycarbonyl.

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[21] **4a**: ^{1}H NMR (600 MHz, D_2O, 330 K): δ = 8.04 (d, 1H), 8.01 (s, 1H), 7.92–7.85 (m, 2H), 7.56 (d, 1H), 7.46 (s, 1H), 7.18–7.15 (m, 2H), 6.87 (s, 1H), 6.86 (s, 1H), 6.25 (s, 2H), 6.16 (s, 1H), 5.95 (s, b, 1H), 5.88 (s, 1H), 5.75 (s, 1H), 5.71 (d, 1H), 5.67 (d, 1H), 5.08 (s, 1H), 4.84 (d, 1H), 4.54 (s, 1H), 4.41 (t, 1H), 4.28–4.22 (m, 3H), 4.19 (t, 1H), 4.13–4.05 (m, 6H), 4.02–3.98 (m, 2H), 3.88 (dd, 1H), 3.17–3.15 (m, 1H), 3.11 (s, 1H), 2.59–2.52 (m, 1H), 2.13–2.12 (m, 1H), 2.05–2.01 (m, 1H), 1.90–1.88 (m, 1H), 1.19 (d, 3H), 1.16 ppm (d, 3H); HRMS (MALDI) *m/z* calcd for $C_{65}H_{72}Cl_2N_8O_{27}Na$ $[M+Na]^+$: 1489.3776, found: 1489.3703; HPLC (C_{18} reverse-phase, 5–80% CH_3CN in H_2O + 0.1% TFA, 0–30 min) t_R = 10.6 min.

