HYCRON, an Allylic Anchor for High-Efficiency Solid Phase Synthesis of Protected Peptides and Glycopeptides

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The recently developed allylic HYCRON anchor[1] exhibits excellent properties for the solid phase synthesis of protected peptides and glycopeptides. Model reactions with analogous low molecular weight compounds assessed the acid- and base-stability of the polar and flexible HYCRON linkage. The new anchor is available in a two-step synthesis and allows the use of both the Boc- and the Fmoc-strategy, which can even be combined within one synthesis. Protected glycopeptides are released under almost neutral conditions, taking advantage of the Pd(0)-catalyzed allyl transfer to a weakly basic nucleophile such as *N*-methylaniline. The highly efficient synthesis of *O*-αGalNAc-(T_N)-peptides of the MUC-1 repeating unit is described. Acid- and base-stability of the allyl ester linkage enabled the synthesis of an *O*-glucosylated peptide by first removing a threonine *tert*-butyl group on the solid phase and subsequently glycosylating the liberated resin-bound hydroxyl component.

Introduction

Proteins and peptides exist in a variety of conjugated forms, the most important being glycoconjugates.[2] N- and O-Glycosidically bound carbohydrates affect the conformation of proteins and regulate their activity and biological half-lives.[3] Glycoproteins participate in biological recognition processes such as cell adhesion, regulation of cell growth, and cell differentiation. During tumor-transformation, the glycosylation pattern of glycoproteins, such as mucins, changes markedly.[4] Mucins are excessively *O*-glycosylated proteins with a carbohydrate portion reaching 50−80%.[5] Mucins expressed from various epithelial cell types in soluble and membrane-associated forms typically consist of several serine- and threonine-rich repeating units which serve as carbohydrate scaffolds. During tumor progression the balance between the mucin-mediated adhesion[6] and antiadhesion processes is no longer under control. Simultaneously, alterations of the glycosylation pattern occur, because certain glycosyltransferases are expressed in lower concentrations.[7] These two phenomena seem to be causally related. For instance, T_N-antigen structures (Ser/Thr-(αGalNAc)) were demonstrated to be tumor-associated and T-antigen structures (Ser/Thr(α-3-(βGal)-GalNAc)) even tumor-specific in breast tissue.[8]

Oligonucleotides[9] and oligopeptides[10] are readily available via solid phase synthesis. The properties of the anchor group positioned between the oligomer to be synthesized and the polymeric support are crucial for the success of a solid phase synthesis. There is an increasing demand for linkage groups which provide an additional orthogonal stability and can, thus, accommodate a wider range of reaction conditions. This particularly holds true for the synthesis of glycopeptides and for the growing field of combinatorial chemistry.[11] The synthesis of glycopeptides and phosphopeptides as well as peptides with acid- and base-labile protecting groups, which are to be employed in fragment condensations,[12] requires anchor groups which allow the release of the target molecule under conditions that leave the labile structures unaffected.

Most of the commonly used anchors for the solid phase synthesis of protected peptides and glycopeptides are either acid-labile[13] or base-labile. Nearly all of the acid-labile anchor groups belong to the benzyl ester type[14] and produce long-lived, stable carbocations during their cleavage. These may cause undesired alkylations of nucleophilic moieties.[15] Furthermore, acid-labile protecting groups such as the *tert*-butyloxycarbonyl(Boc)-group

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(1) Abbreviations: AA, amino acid; Ac, acetyl; Acm, acetamidomethyl; Bn, benzyl; Boc, *tert*-butyloxycarbonyl; DCC, *N,N′*-dicyclohexylcarbodiimide; DIC, *N,N′*-diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DKP, diketopiperazine; EtSMe, ethyl methyl sulfide; Fmoc, 9-fluorenylmethoxycarbonyl; Gal, D-galactopyranose; GalNAc, 2-acetamido-2-deoxy-D-galactopyranose; Glc, D-Glucopyranose; GPC, gel permeation chromatography; HOBt, 1-hydroxybenzotriazole; HONSu, *N*-hydroxysuccinimide; HYCRAM, *hy*droxy*cr*otonoyl (*a*mino*m*ethyl)polystyrene; HYCRON, *hy*droxy*cr*otyl-oligoethylene glycol-*n*-alkanoyl; MPLC, medium performance liquid chromatography; Mtr, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; Muc, mucine; NMM, *N*-methylmorpholine; Pac, phenacyl; PS, polystyrene; tBu, *tert*-butyl; Trt, trityl; TBTU, 2-*O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; Z, Benzyloxycarbonyl.

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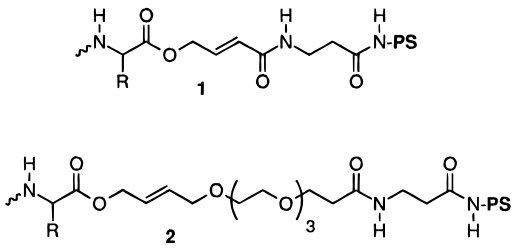
Scheme 1

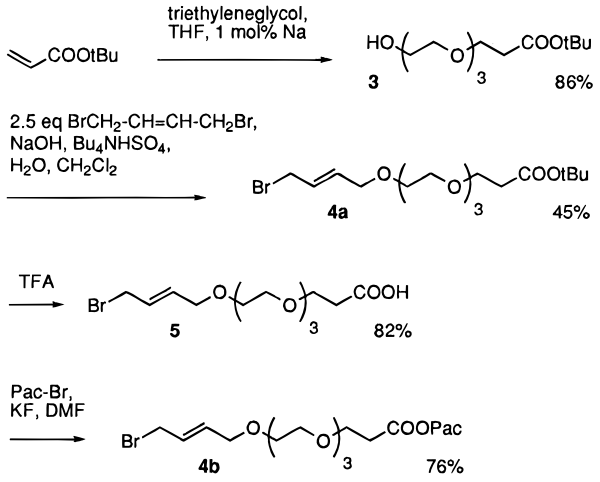


cannot be removed on solid phase without detaching the peptide. Base-labile linkages such as in Kaiser's oxime resin are not compatible with the widely used fluorenylmethoxycarbonyl(Fmoc)-strategy. Most of the existing photolabile linkers can only be cleaved in low yields if longer peptide sequences are synthesized. Photolabile anchors with high cleavability are usually base-sensitive[16] and not fully compatible with Fmoc chemistry.

Linkers of the general allyl-type[17] are of particular value, because they are removable under almost neutral conditions and are orthogonally stable to the commonly used acid- and base-labile protecting groups.[18] Allylic anchoring offers the possibility of applying both the Boc- and the Fmoc-group for temporary *N*-terminal protection in solid phase peptide synthesis. The strategies may even be combined in one synthesis. The cleavage of the allylic linkage is achieved by a palladium(0)-catalyzed transfer reaction[19] of the allyl group to a nucleophile, which acts as an allyl scavenger. Nucleophiles such as morpholine,[20] the less basic *N*-methylaniline,[21] or dimedone[22] irreversibly trap the allylic moiety.

Solid phase peptide synthesis with allylic anchoring was introduced using hydroxycrotonic acid linkers (*β*-HYCRAM **1**, Scheme 1). A range of complex glycopeptides have been successfully synthesized on the *β*-HYCRAM-support.[23] While Boc-strategy provided glycopeptides in high yield, the application of the Fmoc-strategy showed losses of some peptide during the synthesis.[24] Moreover, complete release of the peptide was sometimes difficult to achieve.

Scheme 2



In the design of a new allylic anchoring group, a more flexible spacer was inserted between the anchor and the polymer in order to facilitate an efficient access of the Pd(0) complex during the detachment reaction. The success achieved with craft copolymers[25] of polyethylene glycol and polystyrene inspired the incorporation of a polar spacer of the ethylene glycol type. In addition, in this HYCRON anchor[26] **2** (Scheme 1), the α,*β*-unsaturated carbonyl structure was avoided, since it could be responsible for the occasionally low yields in Fmoc chemistry with *β*-HYCRAM **1**. As in *β*-HYCRAM, *β*-alanine is used as a standard amino acid, since it simplifies the determination of the peptide loading.[27]

Results and Discussion

Synthesis of a Low Molecular Weight Model. The first step in synthesis of an anchor derivative **4**, which allows the coupling of the starting amino acid via nucleophilic esterification consists in the sodium glycolate catalyzed 1,4-addition of triethylene glycol to *tert*-butyl acrylate (Scheme 2). The subsequent reaction of the adduct **3** with 1,4-dibromo-2-butene is carried out under phase transfer conditions, which avoids the retro-Michael reaction leading to unseparable mixtures. This elimination is initiated by abstraction of an α-proton, which is unlikely to occur in the resin-bound molecule, because proton abstraction would take place at the amide function disabling further deprotonation. Treatment of **4a** with TFA removes the *tert*-butyl group.

The carboxylic acid **5** is activated with DCC[28] and *N*-hydroxysuccinimide[29] (HONSu) and reacted with *β*-Ala-NH-Bn to give **6** (Scheme 3). A slight excess of the acid compound must be used in order to prevent the concomitant allylation of HONSu. Compound **6** can be considered a low molecular weight model of an (aminomethyl)-polystyrene (H_2N-PS) resin functionalized with the standard amino acid *β*-alanine and the anchor-bromide. The reaction of the cesium salt of Z-protected alanine

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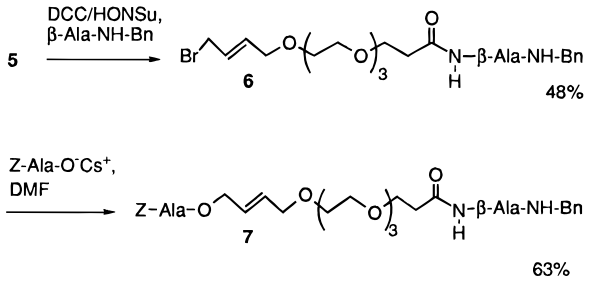
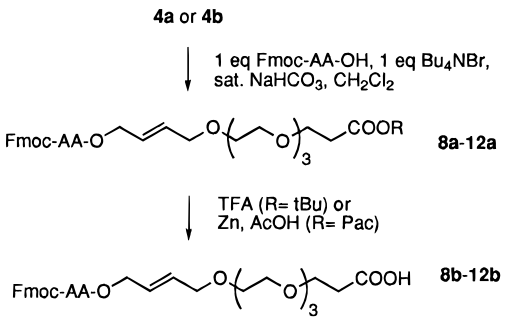
Table 1. Nucleophilic Esterification of Fmoc-Amino Acids with Anchor-Bromides 4a,b and Removal of the C-Terminal Protecting Group (see also Scheme 4)

	Ala (R = tBu), % yield	Gly (R = tBu), % yield	Pro (R = tBu), % yield	Val (R = tBu), % yield	Thr(tBu) (R = Pac), % yield
ester formation	**8a**, 72	**9a**, 65	**10a**, 81	**11a**, 67	**12a**, 83
R removal	**8b**, 84	**9b**, 88	**10b**, 85	**11b**, 57	**12b**, 97

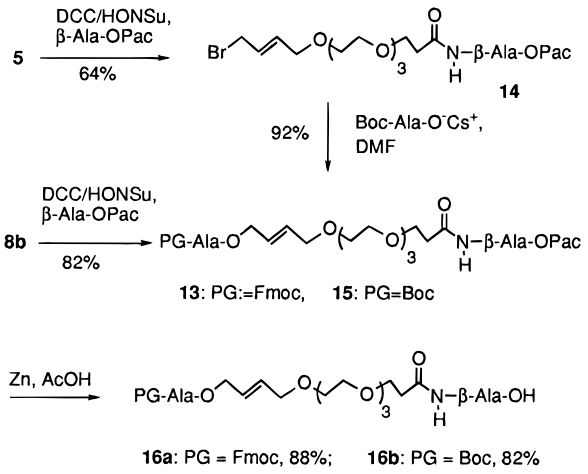
Scheme 3



Scheme 4



Scheme 5



with bromide **6** provides the model compound **7**. This nucleophilic esterification is known to proceed without any detectable racemization.[30] Unfortunately, the chromatographic behavior of **6** and **7** is nearly identical resulting in a small contamination with **6** even after twofold MPLC and preparative HPLC. However, the material is useful for stability tests, since the reactions of both **6** and **7** can be monitored by analytical HPLC. In order to assess the stability of the HYCRON system toward the conditions of *N*-Boc- and *N*-Fmoc removal, compound **7** is treated with dichloromethane/TFA (1:1) and morpholine, respectively. Several reaction cycles are simulated by an exposure of two days. HPLC analysis shows that formation of new substances occurs in an amount of less then 4%. This is a promising result for the application of HYCRON-anchoring in solid phase peptide synthesis.

Synthesis of Anchor Conjugates and Resin Loading. The starting amino acids should be loaded onto the polymer via their anchor conjugates, since attempts to couple amino acid cesium salts to a PS-resin functionalized with β-alanine and the bromo-anchor **5** resulted in low loading yields. Using the linkage agent **4a**, anchor conjugates of Fmoc-amino acids can be synthesized. For conjugate synthesis of amino acids with acid-labile side chain protecting groups, the *tert*-butyl group of **4a** is exchanged (Scheme 2). The phenacyl (Pac) ester **4b** provides orthogonally stable protection to most side chain protecting groups in SPPS.

Usually Fmoc-amino acids are alkylated via the cesium salt method. This reaction is less favorable for the HYCRON-system, because the rate of the alkylation reaction is slow and Fmoc-cleavage competes in the basic environment. In contrast, this nucleophilic esterification with the anchor bromides **4a** and **4b** proceeds mildly under phase transfer conditions using the base $NaHCO_3$ (Scheme 4). Reactants need not to be applied in excess. Conjugates **8a**−**12a** are easily accessible (Table 1) using this method. TFA liberates the carboxylic function in the case of *tert*-butyl protection to give **8b**−**11b**, and zinc powder in acetic acid reductively cleaves the Pac-group from **12a** to form **12b**.

Conjugates consisting of starting amino acid, anchoring group, and standard amino acid are synthesized via two possible routes, which differ in the sequence of condensations. For synthesis of conjugate **16b** containing Boc-Ala, the anchor-bromide **5** is condensed with β-Ala-OPac. Amide **14** is employed in an alkylation of the Boc-Ala cesium salt followed by reductive removal of the Pac-group (Scheme 5). Conjugate **16a** is synthesized by coupling **8b** to β-Ala-OPac and subsequent reductive cleavage of the Pac-ester **13**.

In order to attach the amino acid-anchor-conjugates to the polymer, Boc-β-Ala loaded (aminomethyl)polystyrene (H_2N-PS) is treated with TFA and neutralized with diisopropylethylamine (DIEA). The conjugates **8b**−**12b** are coupled to this resin by the DIC/HOBt[31] method (Scheme 6). The same reaction conditions are applied for binding the conjugates **16a** and **16b** to unmodified H_2N-PS resin. Any amino groups that have not been acylated are capped with acetic anhydride/pyridine. Table 2 shows the loadings and yields achieved by these reactions.

In the previously described synthesis of anchor conjugates the allylic ester is formed by allylation of the carboxylic acid. However, nucleophilic esterifications can be accompanied by alkylations of other nucleophilic structures. For instance, the esterification of Fmoc-Cys(Acm) under the above mentioned conditions proceeds

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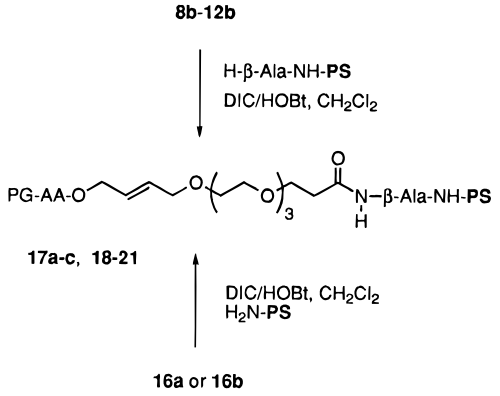
Table 2. Attachment of Amino Acid-Anchor-Conjugates to the Polystyrene Resin (see also Scheme 6)

AA	PG	anchor-conjugate	loading/ (mmol of AA/g), yield[a]
	Fmoc	**8b**	**17a**, 0.52, 89%
Ala	Fmoc	**16a**	**17b**, 0.71, 89%
	Boc	**16b**	**17c**, 0.67, 75%
Gly	Fmoc	**9b**	**18**, 0.50[b], 80%
Pro	Fmoc	**10b**	**19**, 0.52, 61%
Val	Fmoc	**11b**	**20**, 0.41, 75%
Thr(tBu)	Fmoc	**12b**	**21**, 0.40[c], 66%

[a] Yield = $l_{AA}/l_{\beta-Ala} \times 100$ for resins **17a** and **18**−**21**, $l_{AA}/l_{NH_2} \times 100$ **17b**,**c** (l = loading, determined by amino acid analysis). [b] Content of Fmoc group (photometrically). [c] Original loadings and yields are higher, and determination by amino acid analysis gives values that are too low.

Scheme 6



with a concomitant allylation of the thiol function. The side reactions are avoided if the carboxylic group is reacted with the anchor-alcohol. In contrast to the amino acid loadings with HYCRON-bromides as substrates, this procedure can also be performed on the solid support. Acetoxylation of the anchor-bromide **4a** and removal of the tBu-group produces the protected form **23** of the allylic anchor-alcohol (Scheme 7). Using DIC/HOBt, compound **23** is coupled to β-Ala-NH-PS, and **24** is deacetylated by means of aqueous NaOH/dioxane. The attachment of the starting amino acid Fmoc-Cys(Acm) is achieved by activating the C-terminus with 2,6-dichlorobenzoyl chloride[32] *in situ* and then coupling to the resin-bound allylic alcohol in the presence of pyridine. This reaction takes place without affecting the thiol function as was demonstrated by the esterification of compound **26** in solution. It should be taken into account that the application of this more convenient method results in a higher propensity of racemization.

Synthesis of Tri- and Tetrapeptides. In order to investigate the properties of the HYCRON-anchor regarding stability and cleavability, protected tri- and tetrapeptides **28**−**31** have been synthesized (Scheme 8). The coupling reactions were accomplished with a three- to four-fold excess of the N-protected amino acids activated by DIC and HOBt. Temporary *N*-Boc-groups (Scheme 8, PG = Boc) were removed by treating the peptide-resin with dichloromethane/TFA (1:1). Reactions with DMF/morpholine (4:3) for 2 h deprotected the amino groups if the Fmoc-strategy was applied (Scheme 8, PG = Fmoc). Removal of the *N*-Fmoc-groups is usually complete in less than 45 min. However, in these syntheses, the cleavage times were extended in order to

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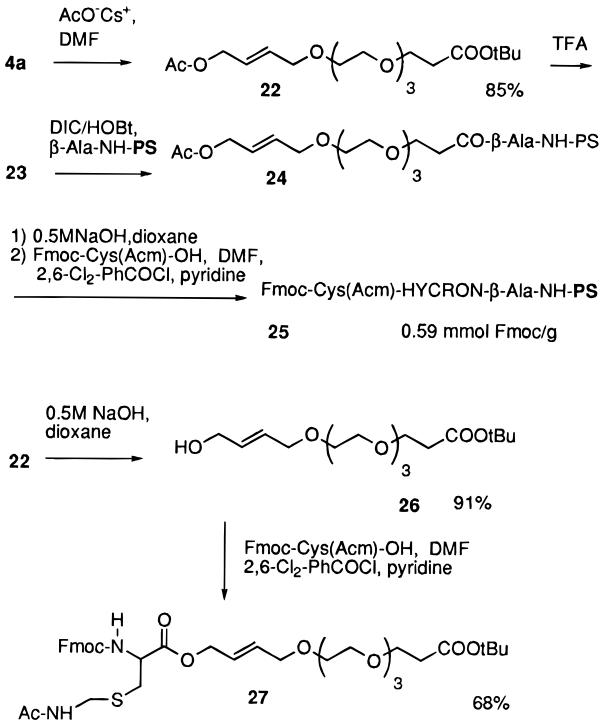
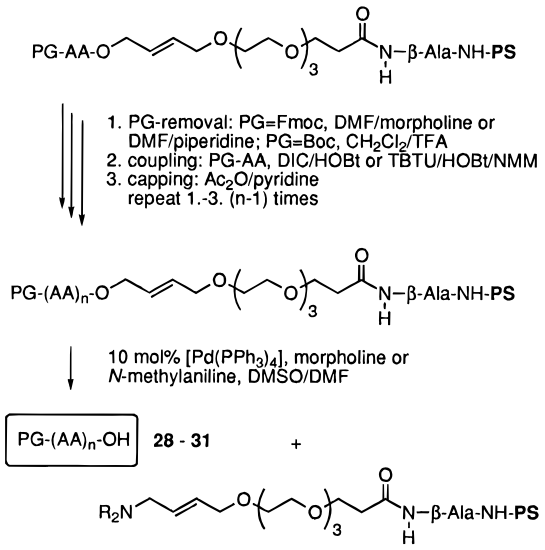
Scheme 7



Scheme 8



facilitate the detection of undesired anchor cleavage (Table 3). The final release of the peptides was achieved by treating the peptide-resin with a catalytic amount of the palladium(0)-catalyst and a ten- to fifteen-fold excess of the scavenger nucleophile under exclusion of oxygen. Morpholine was used for the release of *N*-Boc-protected peptides and *N*-methylaniline for the liberation of *N*-Fmoc protected peptides, since this base does not to cleave the Fmoc-group. Detachment yields were demonstrated to be in the range of 90% on average. Table 3 shows peptide losses, which occur at the stage of the dipeptide-resin due to the formation of diketopiperazines (DKP) and the total peptide losses caused by any other undesired cleavage of the HYCRON-linkage. It is clear that the application of the Boc-strategy (peptide **28**) does not result in any premature losses at all. Fmoc-cleavage with DMF/morpholine (4:3) for 2 h leads to a considerable amount of DKP formation (see peptides **29**−**31**). The

Table 3. Synthesis of Protected Tri- and Tetrapeptides (see also Scheme 9)

28 Boc-Leu-Phe-Ala-OH

29 Fmoc-Leu-Phe-Ala-OH

30 Fmoc-Tyr(tBu)-Leu-Ala-OH

31 Fmoc-Gly-Leu-Tyr(tBu)-Ala-OH

peptide	PG removal	peptide-DKP,[a] % yield	losses, % total[b]	scavenger R_2NH	detachment, % yield[c]
28	CH_2Cl_2/TFA, 1:1, 45 min	0	0	morpholine	89
29	DMF/morpholine, 4:3, 2 h	20	22	*N*-methylaniline	81
30	DMF/morpholine, 4:3, 2 h	38	33	*N*-methylaniline	93
31	DMF/morpholine, 4:3, 2 h	33	34	*N*-methylaniline	96

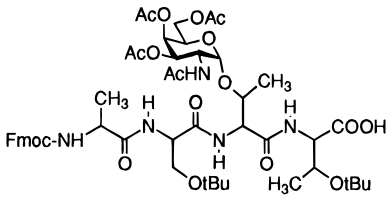
[a] Diketopiperazine formation = $[(l_{Ala}/l_{\beta-Ala})_{tripep-resin}/(l_{Ala}/l_{\beta-Ala})_{dipep-resin} - 1] \times 100$. [b] $((l_{Ala}/l_{\beta-Ala})_{pep-resin}/(l_{Ala}/l_{\beta-Ala})_{AA-resin} - 1) \times 100$. [c] $(1 - [(l_{start-AA}/l_{\beta-Ala})_a/(l_{start-AA}/l_{\beta-Ala})_b] \times 100$, ($l$ = loading, determined by amino acid analysis, a = after cleavage, AA = amino acid, b = before cleavage).

Table 4. Solid Phase Synthesis of Peptide 33 and *O*-Glycopeptides 32, 34, and 35

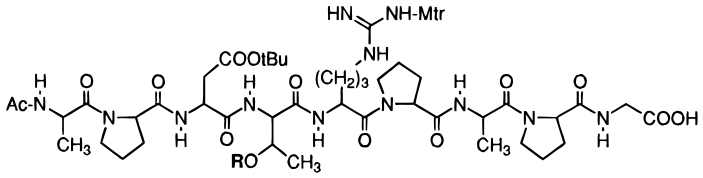
peptide (*n*-mer)	PG removal (PG, conditions)	couplings	detachment, % yield[a]	total, % yield[b]
32 (4-mer)	1.−3.: Fmoc, DMF/morpholine 1:1, 50 min	1.−3.: DIC/HOBt	96	77[c]
33 (9-mer)	1.: Fmoc, DMF/morpholine 1:1, 5 min 2.: Boc, CH_2Cl_2/TFA 1:1, 50 min 3.-9.: Fmoc, DMF/morpholine 1:1, 50 min	1.-8.: DIC/HOBt	87	83
34 (9-mer)	1.: Fmoc, DMF/morpholine 1:1, 50 min 2.: Boc, CH_2Cl_2/TFA 1:1, 50 min 3.−9.: Fmoc, DMF/morpholine 1:1, 50min	1.−4.: DIC/HOBt 5.−6.: TBTU/HOBt/NMM 7.−8.: DIC/HOBt	93	95
35 (20-mer)	1.: Fmoc, DMF/morpholine 1:1, 50 min 2.: Boc, CH_2Cl_2/TFA 1:1, 50 min 3.-19.: Fmoc, DMF/morpholine 1:1, 50min	1.−19.: TBTU/HOBt/NMM	95[d]	45[d]

[a] $(1 - [(l_{start-AA}/l_{\beta-Ala})_a/(l_{start-AA}/l_{\beta-Ala})_b] \times 100$. [b] $n_{peptide}/(m_{start-AA-resin}/l_{start-AA}) \times 100$. [c] Based on loading with β-Ala→minimum yield. [d] Loadings determined photometrically by Fmoc-cleavage (l = load, a = after cleavage, b= before cleavage, n = amount, m = mass).

Scheme 9



Fmoc-Ala-Ser(tBu)-Thr(αAc$_3$GalNAc)-Thr(tBu)-OH **32**

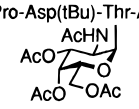


Ac-Ala-Pro-Asp(tBu)-Thr(**R**)-Arg(Mtr)-Pro-Ala-Pro-Gly-OH

33 R= **tBu** , **34** R= **αAc$_3$GalNAc**

Fmoc-Gly-Ser(tBu)-Thr(tBu)-Ala-Pro-Pro-Ala-His(Trt)-Gly-Val-Thr(tBu)-Ser(tBu)-Ala-Pro-Asp(tBu)-Thr-Arg(Mtr)-Pro-Ala-Pro-OH

35



total losses are solely caused by DKP formation since they are identical the DKP losses within the experimental error. No additional losses occur, and it can be concluded that the HYCRON-anchor is orthogonally stable to both the Boc- and the Fmoc-group. The amount of intramolecular aminolysis of the resin-bound dipeptides can be decreased by reducing the Fmoc-cleavage times.

Synthesis of *O*-Glycopeptides. The efficiency of HYCRON-anchoring in solid phase glycopeptide synthesis was demonstrated by the synthesis of the protected *O*-glycotetrapeptide **32** (Scheme 9). Peptide **32** is a glycosylated variant of the N-terminal part of peptide T, a segment of the HIV-envelope glycoprotein gp120.[33] Starting from resin **21**, treatment with DMF/morpholine (1:1) for 50 min removed the Fmoc-group (Table 4). The carbohydrate portion was introduced by coupling the preformed glycosyl amino acid (Fmoc-Thr(αAc$_3$GalNAc)-OH.[34] This, as well as the other coupling reactions, was performed using DIC/HOBt. In the presence of *O*-acetyl groups long coupling times, which may be necessary due to the bulkiness of the glycoside, may result in an acetyl shift to the amino group that is to be acylated. In order to accelerate the elongation reaction of the resin-bound glycodipeptide, the reagents were employed in a larger excess. Release of the *O*-glycosylated tetrapeptide **32** was accomplished in a yield of 96% by palladium(0)-catalyzed transfer of the allyl moiety to *N*-methylaniline. An exact overall yield can not be given. Calculations based on the initial amino acid load is not reasonable because the amino acid analysis of hydroxyamino acids generally gives low values resulting in overestimated yields. As-

(33) (a) Kowalski, M.; Potz, J.; Basiripour, L.; Dorfman, T.; Goh, W. C.; Terwilliger, E.; Dayton, A.; Rosen, G.; Haseltine, W.; Sodroski, J. *Science* **1987**, *237*, 1351. (b) Pert, C. B.; Hill, J. M.; Ruff, M. R.; Berman, R. M.; Robey, W. G.; Arthur, L. O.; Ruscetti, F. W.; Farrar, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 9254.

(34) Kunz, H.; Birnbach, S. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 360.

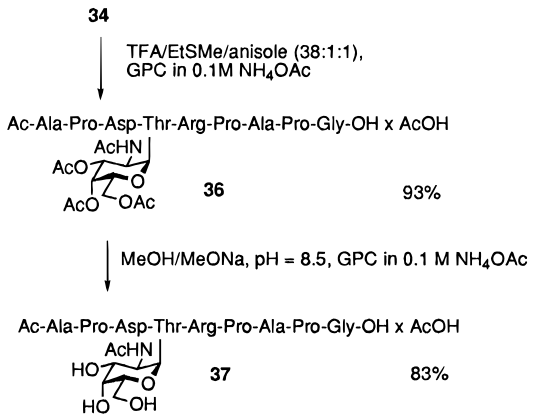
suming a threonine/β-alanine-ratio of 1, which requires a quantitative coupling of **12b** to β-Ala-NH-PS, a minimum overall yield of 77% can be calculated.

Taking advantage of the HYCRON-system, glycopeptide structures of the MUC-1 mucine were synthesized. Synthesis of the protected forms of the nonapeptide **33**, its T_N-variant **34** and of the T_N-eicosapeptide **35**[35] (Scheme 9), which spans the entire 20-mer MUC-1 repeating unit, starts from resins **18** and **19**, respectively. The 20-mer is continuously repeated up to 90 times in the MUC-1 mucin. It is obvious that it would be convenient to couple two 20-mers in order to obtain a dimer of the repeating unit. If possible, a Pro-Gly bond should be formed in fragment condensations, since proline is the amino acid least prone to racemization, and the fastest couplings are achieved when the C-terminal fragment contains N-terminal glycine. For that reason the frame of the repeating unit in peptide **35** is slightly moved to C-terminal proline. Removal of the Fmoc-groups was again accomplished by treatment with DMF/morpholine (1:1) for 50 min. *N*-Boc-amino acids were used for elongation of the amino acid-resins (Table 4). The subsequent acidolysis of the Boc-group reduces the formation of diketopiperazines during cleavage. The strategy was then returned to Fmoc-protection. All acylations were carried out in DMF. For the synthesis of **33** and **34** DIC/HOBt-activation was used except for the couplings of the *O*-glycosyl-threonine and the following aspartic acid building block. These acylations were anticipated to be slow, and therefore the amino acids were activated with TBTU,[36] HOBt, and *N*-methylmorpholine (NMM). Couplings of the *N*-terminal amino acids proline and alanine were each repeated once. The *N*-terminal alanine was acetylated after Fmoc-removal. TBTU-activation was applied for all coupling reactions in the synthesis of T_N-eicosapeptide **35**. Treating the peptide-resins with catalytic amounts of the palladium(0)-complex in the presence of morpholine or *N*-methylaniline in DMSO/DMF detached the peptides in yields between 87 and 95%. Compounds **33**, **34**, and **35** were obtained in overall yields of 83%, 95% and 45% respectively. The overall yields are based on the initial amino acid load determined by amino acid analysis or photometrically after Fmoc-cleavage. In the case of **34** the overall yield is higher than the detachment yield. Of course, this is impossible, but the deviation is within the experimental error of the amino acid analysis.

The *O*-glycononapeptide **34** was deprotected with TFA in the presence of ethyl methyl sulfide and anisole as scavengers[37] (Scheme 10). A sodium methylate-catalyzed transesterification[38] removed the *O*-acetyl groups of the sugar moiety yielding **37**, which was purified by gel permeation chromatography and isolated in an overall yield of 74% relative to the loaded starting amino acid (22 steps).

Removal of Permanent Protecting Groups and O-Glycosylation on Solid Phase. Solid phase synthesis of O-glycopeptides is possible following two routes. The carbohydrate can be introduced by preformed glycosyl amino acids or by glycosylation of an unprotected hydroxyl group on the solid phase. Due to the difficulties involved in the latter method, only very little data have been published so far. Solid phase glycosylation using sugar oxazolines was reported, but unfortunately no yields were given.[39] A yield of 5% was achieved in a silver triflate promoted mannosylation of a serine employing the corresponding glycosyl bromide.[40]

Scheme 10



We had developed a method in which soft electrophiles transform carbohydrates carrying an allylic carbamate at the anomeric position into potent glycosyl donors.[41] In order to examine the efficiency of this glycosylation, a resin-linked peptide with an deblocked hydroxyl group was prepared starting from **20** (Scheme 11). Fmoc-removal was achieved with DMF/piperidine (4:3) in 10 min, and couplings were performed using DIC/HOBt. TFA cleaved the *tert*-butyl ether of the fully protected resin-bound tripeptide. The tripeptide-resin was divided and one part subjected to the palladium(0)-catalyzed allyl transfer to *N*-methylaniline. Tripeptide **38**, a partial structure of MUC-1, was obtained in a total yield of 96%. Before glycosylation, the remaining resin-linked peptide was dried for several days under high vacuum and repeatedly washed with dry dichloromethane. The resin-bound tripeptide with an unprotected hydroxyl group was reacted with 7 equiv of the glucose donor 1-*O*-(*N*-allylcarbamoyl)-2,3,4,6-tetra-*O*-acetylglucose and 2.5 equiv of the promotor *S*-methylbis(methylthio)sulfonium hexachloroantimonate.[41] The large excess of the donor relative to the promotor was used in order to prevent an electrophilic attack of the promotor at the double bond of the anchor system. The glycosylation reaction was repeated twice. After palladium(0)-catalyzed cleavage, the glycosylated Fmoc-tripeptide **39** was isolated in an overall yield of 4%, the unchanged Fmoc-tripeptide **38** in 64%. In the solid phase *O*-glucosylation of a sterically hindered threonine-peptide a yield comparable to the *O*-mannosidation of the serine-peptide described previously was achieved.

Conclusion

The allylic HYCRON-anchoring allows the efficient solid phase synthesis of peptides and glycopeptides in high yields and high purity. The acid- and base-stability

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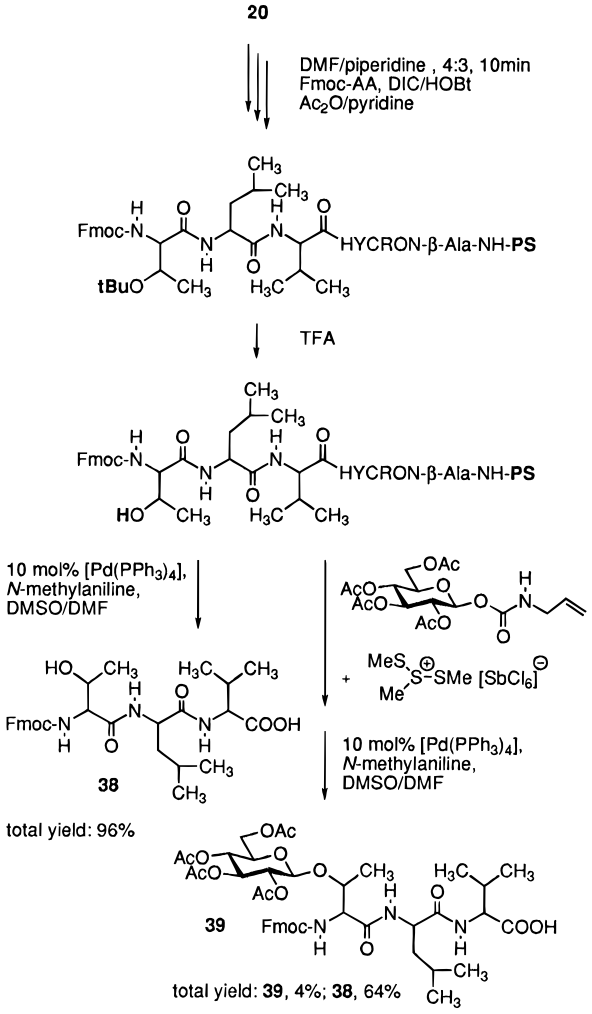
(38) Peters, S.; Bielefeldt, T.; Meldal, M.; Bock, K.; Paulsen, H. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1163.

(39) Hollósi, M.; Kollát, E.; Laczkó, I.; Medzihradszky, K. F.; Thurin, J.; Otvös, L. *Tetrahedron Lett.* **1991**, *32*, 1531.

(40) Andrews, D. M.; Seale, P. W. *Int. J. Pept. Protein Res.* **1993**, *42*, 165.

(41) Kunz, H.; Zimmer; J. *Tetrahedron Lett.* **1993**, *34*, 2907.

Scheme 11



of the HYCRON-anchor makes the application of both the Fmoc- and the Boc-strategy possible. They may even be combined in one synthesis. Peptides and glycopeptides are liberated under almost neutral conditions via the palladium(0)-catalyzed allyl transfer to scavenger nucleophiles such as morpholine or the less basic *N*-methylaniline. The Fmoc-group, the Boc-group, *tert*-butyl-esters, *tert*-butyl ethers, the *O*-acetyl groups in carbohydrates, and particularly *O*-glycosidic bonds are left intact. Therefore, the HYCRON-anchor will be a versatile tool in solid phase synthesis of compounds which demand high standards of orthogonality and also shows properties promising for combinatorial chemistry.

Experimental Section

General.[42] Fmoc-amino acids, (aminomethyl)polystyrene, and Boc-β-Ala-loaded polystyrene were kindly donated by ORPEGEN GmbH, Heidelberg, Germany. THF was freshly distilled from potassium/benzophenone before use. Dichloromethane used in the glycosylation reaction was dried over P_4O_{10} and freshly distilled. Reactions were carried out at room temperature if no specifications are given. Solid phase synthesis was performed manually using a reaction vessel similiar to the Merrifield-reactor.

Amino acid-analysis (AAA) was carried out by ORPEGEN GmbH. Photometric determination of the Fmoc-loadings was performed by treating 1 aliquot of the resin with 2.00 mL of DMF/morpholine (1:1), from which 50 μL was taken after 1 h. This solution was diluted and subjected to UV-analysis (300.5 nm). Chromatography was performed on open columns if no explanations are given in the text. MPLC was performed with silica gel (20−45 μm) and a 430 × 80 mm column. GPC was performed on 950 × 38 mm columns. Some compounds were obtained in form of highly viscous oils, and complete removal of solvents often was impossible. In these cases, yields were calculated including the content of solvent which reaches values up to 5 mass % as determined by NMR-spectroscopy. These corrections are indicated by "cor".

Analytical HPLC was performed on a LKB-system using columns as follows: 1. Eurospher-100, C4/7 μm, 125 × 4 mm (Knauer); 2. Eurospher-100, C4/7 μm, 250 × 8 mm (Knauer); 3. Vydac C4, 250 × 4 mm (Separations Group); 4. Spherisorb ODSII, C18/5 μm, 250 × 4 mm (Bischoff). Gradients used for elution were as follows: 1. 0−42 min: 20% B−100% B in A, 42−48 min: 100% B; 2. 0−28 min: 10% B−50% B in A, 28−48 min: 50% B−100% B in A; 3. 0−2 min: 20% B in A, 2−42 min: 20% B−70% B in A, 42−43 min: 70% B−100% B in A, 43−48 min: 100% B; 4. 0−2 min: 50% B in A, 2−42 min: 50% B−80% B in A; 5. 0−2 min: 10% B in A, 2−42 min: 10% B−40% B in A; 6. 0−2 min: 1% B in A, 2−24 min: 1% B−30% B in A, 24−44 min: 30% B−100% B in A, 44−48 min: 100% B; 7. 0−2 min: 30% B in A, 2−40 min: 30% B−70% B in A, 40−42 min: 70% B−100% B in A, 42−48 min: 100% B. Eluent A: 0.1% TFA in water, eluent B: 0.1% TFA in acetonitrile.

12-Hydroxy-4,7,10-trioxadodecanoic Acid *tert*-Butyl Ester (3). To a solution of anhydrous triethyleneglycol (128.0 mL, 0.94 mol) in 500 mL of THF were added 0.2 g (8.7 mmol) of sodium. When the sodium was dissolved, *tert*-butyl acrylate (48.0 mL, 0.33 mol) was added. The solution was stirred for 20 h and neutralized with 8 mL of 1 M HCl. After removal of the solvent, the residue was suspended in brine and extracted three times with ethyl acetate. The combined organic layers were washed with brine and dried over Na_2SO_4 before the solvent was removed. The resulting colorless oil was dried in vacuo to give 78.7 g (86%) of **3**. 90 MHz ^{1}H-NMR ($CDCl_3$) δ = 3.75−3.50 (m, 14H, $(CH_2CH_2O)_3$, H-3), 2.74 (s, 1H, OH), 2.48 (t, 2H, H-2, J = 6.5), 1.42 (s, 9H).

(*E*)-17-Bromo-4,7,10,13-tetraoxa-15-heptadecenoic Acid *tert*-Butyl Ester (4a). A solution of tetrabutylammonium hydrogensulfate (40.74 g, 0.12 mol) and NaOH (9.75 g, 0.24 mol) in 195 mL of water was added to a mixture of **3** (33.42 g, 0.12 mol) and (*E*)-1,4-dibromo-2-butene (51.6 g, 0.24 mol) in 375 mL of dichloromethane. The two-phase system was vigorously stirred for 45 min. The aqueous layer was separated and extracted twice with 150 mL of dichloromethane. The combined organic layers were concentrated. Addition of 400 mL of diethyl ether led to precipitation of tetrabutylammonium bromide which was separated by filtration. The filtrate was washed twice with brine. The organic layer was dried over $MgSO_4$. After removal of the solvent, chromatography (petroleum ether/EtOAc, 1:1) furnished 22.3 g (45%) of a yellowish oil. 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 5.95−5.69 (m, 2H, H-15, H-16), 3.96 (d, 2H, H-14, J = 4.5), 3.88 (d, 2H, H-17, J = 6.3), 3.65−3.54 (m, 14 H, $(CH_2CH_2O)_3$, H-3), 2.42 (t, 2H, H-2, J = 6.3), 1.36 (s, 9H, tBu). 50.3 MHz ^{13}C-NMR ($CDCl_3$): 170.71 (CO), 131.55 (C-15), 128.45 (C-16), 80.29 (CMe_3), 70.43, 70.33, 70.19 ($(CH_2CH_2O)_3$), 69.53 (C-14), 66.71 (C-3), 36.10 (C-2), 31.82 (C-17), 27.93 (tBu).

(*E*)-17-Bromo-4,7,10,13-tetraoxa-15-heptadecenoic Acid Phenacyl Ester (4b). A solution of **4a** (0.80 g, 1.94 mmol) in 13 mL of TFA was stirred for 1 h. TFA was evaporated. The residue was dissolved in 40 mL of chloroform and washed with 20 mL of 1 M HCl. The aqueous layer was reextracted twice with chloroform. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to yield crude **5**. Then potassium fluoride (0.32 g, 5.5 mmol) and bromoacetophenone (0.45 g, 2.25 mmol) were suspended in DMF (5 mL). A solution of carboxylic acid **5** in DMF (2 mL) was added. The mixture was stirred for 45 min before the precipitate was separated by filtration over Hyflo. The filtrate was diluted with 50 mL of ethyl acetate. The solution was washed twice with saturated $NaHCO_3$-solution. The combined aqueous

(42) See also Eberling, J.; Braun, P.; Kowalczyk, D.; Schultz, M.; Kunz, H. *J. Org. Chem.* **1996**, *61*, 2638.

layers were extracted twice with ethyl acetate. The combined organic layers were washed with brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by chromatography (petroleum ether/EtOAc, 1:2), yielding 0.58 g (63% based on **4a**) of a clear, yellowish, and viscous liquid. 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.89 (dd, 2H, o-Pac, $J_{o,m}$ = 8.3, $J_{o,p}$ = 1.2), 7.59−7.43 (m, 3H, m-Pac, p-Pac), 5.89−5.85 (m, 2H, H-15, H-16), 5.34 (s, 2H, CH_2-Pac), 4.01 (d, 2H, H-14, J = 4.6), 3.93 (d, 2H, H-17, J = 6.3), 3.81 (t, 2H, H-3, J = 6.6), 3.64−3.55 (m, 12 H, $(CH_2CH_2O)_3$), 2.78 (t, 2H, H-2, J = 6.6). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 191.80 (CO-Pac), 170.73 (CO), 133.69 (p-Pac), 133.60 (i-Pac), 131.50 (C-15), 128.65 (o-Pac), 128.37 (C-16), 127.53 (m-Pac), 70.36, 70.25 ($(CH_2CH_2O)_3$), 69.45 (C-14), 66.20, 66.11 (CH_2-Pac, C-3), 34.52 (C-2), 31.86 (C-17). Anal. Calcd for $C_{21}H_{29}BrO_7$: C, 53.29; H, 6.17; Br, 16.88. Found: C, 53.20; H, 6.14; Br, 16.67.

(*E*)-17-Bromo-4,7,10,13-tetraoxa-15-heptadecenoic Acid (5). A solution of **4a** (0.22 g, 3.38 mmol) in TFA (10 mL) was stirred for 45 min. TFA was evaporated in vacuo, and the residue was dissolved in chloroform and washed with 1 M HCl. The aqueous phase was reextracted with chloroform. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Chromatography (EtOAc/AcOH, 100:1) yielded 0.16 g (82%) of a clear, yellowish, and highly viscous oil. 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 5.93−5.68 (m, 2H), 3.95 (d, 2H, J = 4.6), 3.87 (d, 2H), 3.70−3.48 (m, 14H, J = 6.4), 2.53 (t, 2H, J = 6.3). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 175.72, 131.39, 128.51, 70.27, 70.20, 70.18, 69.33, 66.11, 34.57, 31.77. Anal. Calcd for $C_{13}H_{23}BrO_6$: C, 43.96; H, 6.53. Found: C, 43.91; H, 6.51.

(*E*)-17-Bromo-4,7,10,13-tetraoxa-15-heptadecenoyl-*β*-alanine Benzylamide (6). To a solution of the carboxylic acid **5** (0.40 g, 1.13 mmol) in 10 mL of dry ethyl acetate were added HONSu (0.12 g, 1.08 mmol) and DCC (0.22 g, 1.08 mmol) at 0 °C. After stirring for 30 min, the mixture was allowed to warm up to room temperature and was stirred for additional 16 h. The urea which precipitated upon cooling to 0 °C was filtered off. The solvent was removed, and the residue was treated with 20 mL of acetone to complete precipitation of urea. The filtrate was concentrated in vacuo before 5 mL of ethanol was added. A solution of *β*-alanine benzylamide (0.20 g, 1.13 mmol) in 6 mL of ethanol/water (2:1) was added in portions. The solvents were removed after 3 h of stirring. The residue was purified by chromatography (petroleum ether/EtOAc, 1:1, elution of 0.13 g starting material with EtOAc, elution of product with EtOAc/EtOH, 1:1). A 0.28 g (48%) amount of a yellowish, highly viscous oil was obtained. 200 MHz ^{1}H-NMR (DMSO-d_6) δ = 8.41 (bs, 1H), 8.38 (bs, 1H), 7.34−7.21 (m, 5H), 5.96−5.68 (m, 2H), 4.27−3.82 (m, 6H), 3.57−3.47 (m, 14H), 3.27 (dt, 2H), 2.35−2.27 (m, 4H). 50.3 MHz ^{13}C-NMR (DMSO-d_6) δ = 170.26, 169.99, 139.43, 131.56, 128.16, 127.54, 127.11, 126.62, 70.25, 69.71, 69.61, 69.45, 69.06, 68.74, 66.73, 41.93 39.83, 35.98, 35.24.

(*E*)-17-(*N*-(Benzyloxycarbonyl)-L-alanyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-*β*-alanine Benzylamide (7). To a stirred solution of Z-alanine (1.66 g, 7.46 mmol) in 50 mL of methanol was added Cs_2CO_3 (1.21 g, 3.73 mmol). The solvent was removed after 45 min. The residue was coevaporated three times with toluene. The salt was dried in vacuo and dissolved in 30 mL of DMF. A solution of **6** (3.5 g, 6.79 mmol) in DMF (20 mL) was added. After 18 h of stirring, the mixture was filtered over Hyflo. The filtrate was concentrated in vacuo and the residue dissolved in 200 mL of chloroform. The solution was extracted twice with 80 mL of saturated $NaHCO_3$-solution. The aqueous layers were extracted with 50 mL of chloroform, and the combined organic layers were dried over Na_2SO_4. After removal of the solvent, 5.1 g of raw material were obtained. Purification by chromatography (EtOAc/EtOH) yielded 3.99 g of a product which was further purified by MPLC (EtOAc/EtOH 20:1). A second MPLC-purification (EtOAc/EtOH 40:1) furnished 2.80 g (63%) of a colorless, highly viscous oil with a purity of 80% as determined by HPLC.

A small amount was further purified by preparative HPLC (Spherisorb ODSII, C18/5 μm, 250 × 40 mm, 45% H_2O in acetonitrile, 20 mL/min) in order to obtain material sufficiently pure for stability tests. 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.28−7.18 (m, 11H, H_{ar}, NH-Bn), 6.99 (m, 1H, NH-*β*-Ala), 5.77−5.68 (m, 3H, H-15, H-16, NH-Ala), 5.03 (s, 2H, CH_2-Z), 4.55 (d, 2H, H-17, J = 3.5), 4.35−4.28 (m, 3H, A^{α}, CH_2-Bn), 3.93 (d, 2H, H-14, J = 3.3), 3.64−3.40 (m, 16H, $(CH_2CH_2O)_3$, *β*-Ala^{β}, H-3), 2.37, 2.35 (2 × t, 4H, *β*-Ala^{α}, H-2, J_1 = 6.2, J_2 = 5.8), 1.35 (d, 3H, A^{β}, J = 7.2). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 172.51, 171.56, 171.16, 155.52 (CO-Z), 138.39, 136.24 (i-Bn, i-Z), 131.25, 128.39, 128.31, 127.91, 127.81, 127.59, 127.13 (C-15, o-,m-,p-Bn, o-,m-,p-Z), 125.73 (C-16), 70.51, 70.43, 70.34, 70.16, 70.05, 69.50 (C-14), 66.97 (C-3), 64.84 (C-17), 49.63 (A^{α}), 43.26 (CH_2-Bzl), [36.75, 35.79, 35.55 (C-2, *β*-Ala^{α}, *β*-Ala^{β})], 18.29 (A^{β}). Anal. Calcd for $C_{34}H_{47}O_{10}N_3$: C, 58.08; H, 6.53; N,6.17. Found: C, 57.84; H, 5.86; N, 6.17.

General Procedure for Synthesis of Anchor Conjugates 8a−12a. A 0.25 molar solution of 1 equiv of the Fmoc-amino acid and 1 equiv of the "anchor-bromide" **4a** or **4b** in dichloromethane was added to a solution consisting of 1 equiv of tetrabutylammonium bromide in a volume of aqueous saturated $NaHCO_3$ which equals the volume of the organic solvent. The two-phase system was stirred until TLC monitoring showed the completion of the reaction (2−18 h). The organic layer is separated and the aqueous phase extracted twice with dichloromethane. The combined organic layers were dried over $MgSO_4$, and solvents were evaporated in vacuo. Further purification is accomplished by chromatography.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-alanyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid *tert*-Butyl Ester (8a). Yield: 72% (cor). R_f: 0.30 (petroleum ether/EtOAc, 1:2). $[\alpha]^{22}_D$ = −11.6 (c = 1, MeOH). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.72 (d, 2H), 7.56 (d, 2H), 7.39−7.22 (m, 4H), 5.82−5.77 (m, 2H, H-15, H-16), 5.55 (d, 1H, A^{NH}, J = 7.7), 4.60 (d, 2H, H-17, J = 3.8), 4.40−4.34 (m, 3H, A^{α}, CH_2-Fmoc), 4.17 (t, 1H, H-9-Fmoc, J = 6.9), 3.97 (d, 2H, H-14, J = 3.4), 3.67 (t, 2H, H-3, J = 6.6), 3.60−3.56 (m, 12H), 2.46 (t, 2H, H-2, J = 6.6), 1.41−1.38 (m, 12H, tBu, A^{β}). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 172.51, 170.68, [155.51, 143.77, 143.64, 141.1 (Fmoc)], 131.46 (C-15), [127.51, 126.88 (Fmoc)], 125.59 (C-16), [124.88, 119.77 (Fmoc)], 80.26 (CMe_3), 70.51, 70.43, 70.40, 70.31, 70.17 ($(CH_2CH_2O)_3$), 69.54 (C-14), 66.78, 66.71 (CH_2-Fmoc, C-3), 64.96 (C-17), 49.54 (A^{α}), 47.01 (C-9-Fmoc), 36.12 (C-2), 27.92 (tBu), 18.39 (A^{β}).

(*E*)-17-(*N*-9-(Fluorenylmethoxycarbonyl)glycyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid *tert*-Butyl Ester (9a). Yield: 65%. R_f: 0.33 (petroleum ether/EtOAc, 1:2). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.74 (d, 2H), 7.58 (d, 2H), 7.41−7.25 (m, 4H), 5.91−5.79 (m, 2H), 5.37 (d, 1H, G^{NH}, J = 5.4), 4.63 (d, 2H, H-17, J = 4.3), 4.38 (d, 2H, CH_2-Fmoc, J = 7.0), 4.21 (t, 1H, J = 7.0), 4.00−3.87 (m, 4H, H-14, G^{α}), 3.71−3.54 (m, 14H), 2.47 (t, 2H, J = 6.5), 1.42 (s, 9H). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 170.72, 169.58, 156.21, 143.62, 141.07, 131.60, 127.51, 126.88, 125.50, 124.89, 119.77, 80.30, 70.47, 70.37, 70.28, 70.14, 69.50, 66.92, 66.68, 64.95, 46.90, 42.54 (G^{α}), 36.06, 27.90.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-prolyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid *tert*-Butyl Ester (10a). Yield: 81% (cor). R_f: 0.35 (petroleum ether/EtOAc, 1:1). $[\alpha]^{22}_D$ = −20.0 (c = 0.88, $CHCl_3$). 400 MHz ^{1}H-NMR ($CDCl_3$, signal doubling due to Pro-(*E*,*Z*)-rotamers) δ = 7.73, 7.72 (2x d, 2H), 7.61−7.51 (m, 2H), 7.39−7.40 (m, 2H), 7.30−7.25 (m, 2H), 5.84−5.71 (m, 2H), 4.62−4.52 (m, 2H), 4.44−4.22 (m, 4H, P^{α}, CH_2-Fmoc, H-9-Fmoc), 3.97 (d, 1.1H, H-14, J = 4.6), 3.88 (d, 0.9H, H-14, J = 5.0), 3.68−3.43 (m, 16H), 2.47 (t, 2H, J = 6.5), 2.27−2.19 (m, 1H, $P^{\beta 1}$), 2.04−1.85 (m, 3H, $P^{\beta 2}$, P^{γ}), 1.41 (s, 9H). 100.6 MHz ^{13}C-NMR ($CDCl_3$, signal doubling due to Pro-(*E*,*Z*)-rotamers) δ = 172.22, 172.15, 170.77, 154.76, 144.16, 144.12, 143.86, 143.72, 141.23, 131.49, 131.14, 127.56, 126.97, 126.16, 125.77, 125.13, 125.03, 124.92, 119.86, 80.35, 70.72, 70.52, 70.44, 70.30, 69.60, 67.40, 66.84, 64.77, [59.23, 58.80 (2 × P^{α})], 47.29, 47.20, [46.92, 46.40 (2 × P^{δ})], 36.25, [31.00, 29.82 (2 × P^{β})], 28.04, [24.29, 23.31 (2 × P^{γ})].

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-valyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid *tert*-Butyl Ester (11a). Yield: 67%. R_f: 0.42 (petroleum ether/EtOAc, 1:1). $[\alpha]^{22}_D$ = −2.3 (c = 1.10, $CHCl_3$). 200 MHz ^{1}H-NMR ($CDCl_3$) δ

= 7.71 (d, 2H), 7.57 (d, 2H), 7.39−7.30 (m, 4H), 5.84−5.77 (m, 2H), 5.55 (d, 1H, V^{NH}, J = 9.1), 4.60 (d, 2H, J = 4.1), 4.38−4.19 (m, 4H, V^{α}, CH_2-, H-9-Fmoc), 3.97 (d, 2H, J = 3.6), 3.67 (t, 2H, J = 6.6), 3.60−3.57 (m, 12H), 2.46 (t, 2H, J = 6.6), 2.16 (m_c, 1H, V^{β}), 1.41 (s, 9H), 0.94, 0.87 (2 × d, 6H, $V^{\gamma a}$, $V^{\gamma b}$, J_a = J_b = 6.8). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 171.50, 170.57, 156.00, 143.64, 143.45, 140.97, 131.49, 127.39, 126.77, 125.50, 124.80, 119.66, 80.10, 70.37, 70.27, 70.18, 70.05, 69.37, 66.67, 66.57, 64.69, 58.80 (V^{α}), 46.89, 35.96, 30.94 (V^{β}), 27.79, 18.75 ($V^{\gamma a}$), 17.33 ($V^{\gamma b}$).

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-threonyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid Phenacyl Ester (12a). Yield: 83% (cor). R_f: 0.32 (petroleum ether/EtOAc, 1:2). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.88 (dd, 2H, o-Pac), 7.74 (d, 2H, J = 7.3), 7.65−7.24 (m, 9H, H-1-, H-8-, H-2-, H-7-, H-3-, H-6-Fmoc, m-, p-Pac), 5.86−5.81 (m, 2H), 5.61 (d, 1H, T^{NH}), 5.33 (s, 2H), 4.64−4.22 (m, 7H, T^{β}, H-17, T^{α}, CH_2-, H-9-Fmoc), 3.99 (d, 2H), 3.80 (t, 2H, J = 6.6), 3.63−3.55 (m, 12H), 2.78 (t, 2H, J = 6.6), 1.22 (d, 3H, T^{γ}, J = 6.6), 1.11 (s, 9H).

General Procedure for Synthesis of Anchor-Conjugates 8b−11b. The *tert*-butyl esters **8a−11a** were treated with TFA for 45 min. After evaporation of TFA in vacuo, the residue was dissolved in chloroform. The solution was washed with 1 M HCl. The aqueous phase was extracted twice with chloroform. The combined organic layers were washed with brine and dried over Na_2SO_4 prior to removal of the solvent. The residue is purified by chromatography. The eluents usually contain small amounts of acetic acid, which were removed by coevaporation with toluene.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-alanyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid (8b). Yield: 84% (cor). R_f: 0.27 (EtOAc/AcOH, 100:1). $[\alpha]^{22}_D$ = 6.0 (c = 1.00, toluene). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.75 (d, 2H), 7.59 (d, 2H), 7.43−7.14 (m, 4H), 5.94−5.80 (m, 2H), 5.47 (d, 1H, J = 7.7), 4.64 (d, 2H, J = 3.7), 4.43−4.30 (m, 3H, A^{α}, CH_2-Fmoc), 4.21 (t, 1H, J = 6.8), 4.02 (d, 2H, J = 3.3), 3.75 (t, 2H, H-3, J = 6.2), 3.65−3.52 (m, 12H), 2.60 (t, 2H, H-2, J = 6.2), 1.43 (d, 3H, A^{β}, J = 7.2). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 175.25 (COOH), 172.69 (A^{CO}), 155.62, 143.73, 143.58, 141.10, 131.26, 127.54, 126.91, 125.79, 124.91, 119.81, 70.52, 70.37, 70.28, 70.20, 70.13, 69.45, 66.82, 66.27, 65.02, 49.52 (A^{α}), 46.93, 34.70 (C-2), 18.38 (A^{β}).

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)glycyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid (9b). Yield: 88%. R_f: 0.37 (EtOAc/AcOH, 100:1). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.73 (d, 2H), 7.58 (d, 2H), 7.41−7.24 (m, 4H), 5.85−5.78 (m, 2H), 5.50 (d, 1H, G^{NH}, J = 5.5), 4.63 (d, 2H, J = 4.1), 4.38 (d, 2H, J = 7.0), 4.20 (t, 1H, J = 7.0), 4.00−3.90 (m, 4H, H-14, G^{α}), 3.75 (t, 2H, H-3, J = 6.2), 3.61−3.54 (m, 12H), 2.58 (t, 2H, H-2, J = 6.2). 100.6 MHz ^{13}C-NMR ($CDCl_3$) δ = 175.03 (COOH), 169.74 (G^{CO}), 156.34, 143.68, 141.12, 131.45, 127.56, 126.93, 125.72, 124.94, 119.81, 70.49, 70.37, 70.29, 70.19, 70.14, 69.48, 67.02, 66.32, 64.97, 46.96, 42.59 (G^{α}), 34.72 (C-2).

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-prolyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid (10b). Yield: 85% (cor). R_f: 0.25 (EtOAc/AcOH, 100:1). $[\alpha]^{22}_D$ = −15.83 (c = 1.05, toluene). 200 MHz ^{1}H-NMR ($CDCl_3$, signal doubling due to Pro-(*E*,*Z*)-isomers) δ = 7.74 (d, 2H), 7.60−7.56 (m, 2H), 7.42−7.03 (m, 4H), 5.83−5.75 (m, 2H), 4.63, 4.54 (2 × d, 2H, H-17, J_1 = 3.4, J_2 = 4.4), 4.44−4.28 (m, 4H, P^{α}, CH_2-, H-9-Fmoc), 4.01 (d, 1.1H, H-14, J = 3.9), 3.91 (d, 0.9H, H-14, J = 4.3), 3.74 (t, 2H, H-3, J = 6.2), 3.62−3.51 (m, 14H, P^{δ}, $(CH_2CH_2O)_3$), 2.60 (t, 2H, H-2, J = 6.2), 2.32−2.15 (m, 1H, $P^{\beta 1}$), 2.08−1.96 (m, 3H, $P^{\beta 2}$, P^{γ}). 100.6 MHz ^{13}C-NMR ($CDCl_3$, signal doubling due to Pro-(*E*,*Z*)-isomers) δ = 175.30 (COOH), 172.12 (P^{CO}), 154.82, 154.39, 143.94, 143.67, 143.54, 141.10, 131.15, 130.80, 128.85, 128.05, 127.52, 126.88, 126,29, 125.90, 125.12, 125.02, 119.79, 70.61, 70.48, 70.33, 70.19, 70.11, 69.33, 67.41, 66.26, 64.74, [59.10, 58.70 (2 × P^{α})], 47.09, 47.01, [46.84, 46.34 (2 × P^{δ})], 34.68 (C-2), [30.88, 29.74 (2 × P^{β})], [24.18, 23.21 (2 × P^{γ})].

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-valyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid (11b). Yield: 57%. R_f: 0.39 (EtOAc/AcOH, 100:1). ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 7.74 (d, 2H), 7.58 (d, 2H), 7.41−7.13 (m, 5H), 5.86−5.80 (m, 2H), 5.40 (d, 1H, V^{NH}, J = 9.2), 4.62 (d, 2H, J = 4.2), 4.40−4.17 (m, 4H, V^{α}, CH_2-, H-9-Fmoc), 4.01 (d, 2H, J = 3.8), 3.74 (t, 2H, H-3, J = 6.2), 3.62−3.57 (m, 12H), 2.60 (t, 2H, H-2, J = 6.2), 2.33−2.06 (m, 1H, V^{β}), 0.95, 0.88 (2 × d, 6H, $V^{\gamma a}$, $V^{\gamma b}$, J_a = J_b = 6.8).

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-threonyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid (12b). To a stirred solution of **12a** (2.62 g, 3.13 mmol)$_{cor}$ in 40 mL of glacial acetic acid was added 3.5 g of freshly activated zinc. After 2 h zinc was removed by filtration over Hyflo followed by distillation of the filtrate. The residue was dissolved in dichloromethane and washed with 0.5 M HCl. The aqueous layer was reextracted with dichloromethane. The combined organic layers were dried over $MgSO_4$, and the solvent was removed by distillation. The residue was purified by chromatography (EtOAc/AcOH, 100:1). Coevaporation with toluene yielded 2.11 g (97%, cor) of a clear and colorless, highly viscous oil. R_f: 0.11 (EtOAc/AcOH, 100:1). $[\alpha]^{22}_D$ = 1.54 (c = 1.0, toluene). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.74 (d, 2H, J = 7.3), 7.65−7.59 (m, 2H), 7.41−7.25 (m, 4H), 5.86−5.80 (m, 2H), 5.68 (d, 1H, T^{NH}, J = 9.6), 4.65−4.22 (m, 7H, T^{β}, H-17, T^{α}, CH_2-, H-9-Fmoc), 4.00 (d, 2H, J = 3.6), 3.73 (t, 2H, J = 6.3), 3.67−3.59 (m, 12H), 2.60 (t, 2H, H-2, J = 6.3), 1.22 (d, 3H, T^{γ}, J = 6.2), 1.12 (s, 9H). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 175.03, 170.64 (T^{CO}), 156.63, 143.74, 143.49, 140.98, 131.37, 127.43, 126.83, 125.64, 124.98, 124.92, 119.68, 73.89, 70.42, 70.26, 70.18, 70.06, 69.34, 67.14 (T^{β}), 67.00, 66.26, 64.94, 59.66 (T^{α}), 46.88, 34.53 (C-2), 28.12, 20.63 (T^{γ}).

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-alanyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-*β*-alanine Phenacyl Ester (13). A solution of DCC (1.06 g, 5.14 mmol) in 10 mL of dichloromethane was added to a solution of **8b** (3.53 g, 4.67 mmol)$_{korr}$ and HONSu (0.54 g, 4.70 mmol) in 25 mL of dichloromethane and was stirred for 25 min. The resulting solution was combined with a mixture of *β*-alanine phenacyl ester hydrochloride (1.13 g, 4.67 mmol) and diisopropylethylamine (DIEA, 0.80 mL, 4.67 mmol) in 20 mL of dry ethyl acetate, which had been stirred for 15 min. The urea precipitated after 17 h and was filtered off. The filtrate was concentrated in vacuo. The residue was dissolved in 100 mL of acetone and cooled to 0 °C for 16 h. The mixture was filtered again prior to removal of the solvent in vacuo. The residue was dissolved in chloroform and washed with saturated $NaHCO_3$ and 1 M HCl solution and brine. After each extraction the aqueous layers were reextracted two to three times. The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by chromatography (EtOAc/EtOH, 9:1), which yielded 3.17 g (82%, cor) of a clear, yellowish and viscous oil. R_f: 0.29 (EtOAc/EtOH, 9:1). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.88 (dd, 2H), 7.74 (d, 2H, J = 7.7), 7.60−7.23 (m, 9H), 7.11 (m, 1H, *β*-Ala^{NH}), 5.84−5.79 (m, 2H), 5.41 (d, 1H, A^{NH}), 5.37 (s, 2H), 4.62 (d, 2H), 4.41−4.34 (m, 3H, A^{α}, CH_2-Fmoc), 4.21 (t, 1H, J = 6.9), 3.98 (d, 2H, J = 3.5), 3.74 (t, 2H, J = 6.0), 3.65−3.56 (m, 14H, $(CH_2CH_2O)_3$, *β*-Ala^{β}), 2.68 (t, 2H, *β*-Ala^{α}, J = 6.0), 2.50 (t, 2H, H-2, J = 6.0), 1.42 (d, 3H, A^{β}, J = 7.2). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 192.10, 172.50, 171.32, 155.50, 143.68, 143.53, 141.00, 133.87, 133.62, 131.23, 128.68, 127.52, 127.46, 126.83, 125.59, 124.83, 119.72, 70.44, 70.30, 70.25, 70.07, 69.99, 69.85, 69.40 (C-14), 66.90, 66.67, 65.86, 64.88, 49.45, 46.85, [36.68, 34.79, 34.04 (C-2, *β*-Ala^{α}, *β*-Ala^{β})], 18.24.

(*E*)-17-Bromo-4,7,10,13-tetraoxa-15-heptadecenoyl-*β*-alanine Phenacyl Ester (14). To a solution of **5** (9.74 g, 27.42 mmol) in 150 mL of dichloromethane HONSu (3.15 g, 27.37 mmol) was added. At 0 °C DCC (5.94 g, 28.79 mmol) was added. After stirring for 30 min, the mixture was allowed to warm up to room temperature. Urea was removed by filtration. For completion of precipitation the filtrate was concentrated in vacuo and triturated with acetone. After 14 h at 0 °C the solution was filtered and concentrated to dryness. The residue was dissolved in a mixture of ethyl acetate (190 mL) and ethanol (50 mL). The resulting solution was combined with a mixture of *β*-alanine phenacyl ester hydrochloride (6.67 g, 27.4 mmol) and DIEA (4.7 mL, 27.4 mmol) in 160 mL of ethyl acetate, which already had been stirred for 10 min.

After 3 h the solvents were evaporated. The residue was dissolved in chloroform and washed twice with 0.5 M HCl. The aqueous layers were reextracted with chloroform. The combined organic layers were washed with saturated $NaHCO_3$ solution and brine and dried over Na_2SO_4 before the solvent was removed in vacuo. Chromatography (EtOAc/EtOH, 8:1) yielded 9.5 g (64%) of a clear and colorless oil. R_f: 0.25 (EE:EtOH, 8:1). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.81 (dd, 2H, $J_{o,m}$ = 8.3, $J_{o,p}$ = 1.2), 7.57−7.36 (m, 3H), 7.11 (t, 1H, NH-β-Ala, J = 5.5), 5.80−5.73 (m, 2H), 5.30 (s, 2H), 3.95−3.82 (m, 4H, H-14, H-17), 3.64 (t, 2H, H-3, J = 6.1), 3.55−3.32 (m, 14 H, $(CH_2CH_2O)_3$, β-Ala$^\beta$), 2.60 (t, 2H, β-Ala$^\alpha$, J = 6.2), 2.41 (t, 2H, H-2, J = 6.1). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 192.10, 171.30, 133.88, 133.60, 130.88 (C-15), 128.67, 127.99 (C-16), 127.51, 70.21, 70.04, 69.95, 69.34 (C-14), [66.90, 65.85 (Pac-CH_2, C-3)], [36.64, 34.74, 33.99 (C-2, β-Ala$^\alpha$, β-Ala$^\beta$, C-17)].

(*E*)-17-(*N*-(*tert*-Butyloxycarbonyl)-L-alanyloxy)-4,7,10,-13-tetraoxa-15-heptadecenoyl-β-alanine Phenacyl Ester (15). A solution of Boc-Ala-OH (3.62 g, 19.74 mmol) and Cs_2-CO_3 (3.12 g, 9.57 mmol) in 100 mL of methanol was stirred for 45 min. After removal of methanol in vacuo, the residue was coevaporated with toluene. The dry salt was dissolved in 120 mL of dry DMF, and 3.47 g (6.37 mmol) of bromide **14** was added. After 18 h of stirring, cesium bromide was removed by filtration over Hyflo. The filtrate was concentrated in vacuo. The residue was dissolved in chloroform and washed twice with saturated $NaHCO_3$ solution. The combined aqueous phases were reextracted with chloroform. After drying the combined organic layers over Na_2SO_4, the solvents were evaporated. Chromatography (EtOAc/EtOH, 8:1) furnished 3.84 g (92%) of a clear, yellowish, and highly viscous oil. R_f: 0.34 (EE:EtOH, 6:1). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.80 (dd, 2H), 7.56−7.35 (m, 3H), 7.11 (t, 1H, J = 5.8), 5.74−5.67 (m, 2H), 5.30 (s, 2H), 5.20 (d, 1H, A^{NH}, J = 8.5), 4.50 (d, 2H, H-17, J = 4.2), 4.18−3.97 (m, 1H, A$^\alpha$), 3.89 (d, 2H, H-14, J = 3.7), 3.64 (t, 2H, J = 6.1), 3.54−3.43 (m, 14H), 2.59 (t, 2H, J = 6.2), 2.40 (t, 2H, J = 6.1), 1.32 (s, 9H), 1.26 (d, 3H, A$^\beta$, J = 7.2). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 192.06, 172.72, 171.23, 133.83, 133.60, 130.94, 128.63, 127.48, 125.69 (C-16), 79.37, 70.40, 70.18, 70.01, 69.92, 69.31, 66.87, 65.82, 64.59 (C-17), 48.95 (A$^\alpha$), 36.60, 34.71, 33.95, 28.00 (Boc), 18.14 (A$^\beta$).

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-alanyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanine (16a). The Pac-ester **13** (2.97 g, 3.6 mmol$_{cor}$) was dissolved in 45 mL of glacial acetic acid. After addition of 3.76 g (57.5 mmol) of activated zinc the suspension was stirred for 2.5 h. Workup was performed as described in synthesis of **12b**. The chromatographic purification (EtOAc/EtOH/AcOH, 4:1:0.1) yielded 2.27 g (88%, cor) of a clear, yellowish, and highly viscous oil. R_f: 0.18 (EtOAc/EtOH/AcOH, 4:1:0.05). $[\alpha]^{22}_D$ = −0.93 (c = 1.25, EE). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.75 (d, 2H, J = 7.1), 7.59 (d, 2H, J = 8.5), 7.42−7.26 (m, 4H), 7.08 (t, 1H, β-Ala^{NH}, J = 8.4), 5.84−5.78 (m, 2H), 5.55 (d, 1H, J = 7.8), 4.63 (d, 2H, J = 3.3), 4.43−4.30 (m, 3H), 4.21 (t, 1H, J = 6.9), 4.00 (d, 2H, J = 3.0), 3.70−3.46 (m, 16H, $(CH_2CH_2O)_3$, H-3, β-Ala$^\beta$), 2.53 (t, 2H, β-Ala$^\alpha$, J = 6.0), 2.45 (t, 2H, J = 5.7), 1.43 (d, 3H, J = 7.1). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 174.26 (COOH), 172.69, 172.05, 155.66, 143.74, 143.59, 141.11, 131.12, 127.57, 126.93, 125.89, 124.94, 119.83, 70.51, 70.40, 70.18, 69.35, 67.08, 66.83, 64.98, 49.54, 46.94, [36.63, 34.81, 33.91 (C-2, β-Ala$^\alpha$, β-Ala$^\beta$)], 18.36.

(*E*)-17-(*N*-(*tert*-Butyloxycarbonyl)-L-alanyloxy)-4,7,10,-13-tetraoxa-15-hepta-decenoyl-β-alanine (16b). To a solution of **15** (5.9 g, 9.0 mmol) in 90 mL of glacial acetic acid freshly activated zinc (9.4 g, 0.144 mol) was added. The suspension was stirred for 3 h. Work-up was performed as described in synthesis of **12b**. In addition, the combined organic layers were washed with brine, which after extraction was reextracted four times with small portions of chloroform. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification by chromatography (EtOAc/EtOH/AcOH, 4:1:0.05) yielded 4.09 g (82%, cor) of a clear, colorless, and highly viscous oil. R_f: 0.19 (EtOAc/EtOH/AcOH, 4:1:0.05). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.02 (m, 1H, β-Ala^{NH}), 5.82−5.76 (m, 2H), 5.20 (d, 1H), 4.59 (d, 2H, J = 3.9), 4.27−4.23 (m, 1H, A$^\alpha$), 3.98 (d, 2H, J = 3.5), 3.69−3.58 (m, 14H, $(CH_2CH_2O)_3$, H-3), 3.48 (q, 2H, β-Ala$^\beta$, $J_{\beta,\alpha} = J_{\beta,NH}$ = 6.1), 2.50 (t, 2H, β-Ala$^\alpha$, J = 6.2), 2.43 (t, 2H, J = 5.8), 1.39 (s, 9H), 1.34 (d, 3H, J = 7.1). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 174.33 (COOH), 172.89, 172.24, 130.92, 125.95, 79.37, 70.49, 70.12, 69.31, 66.96, 64.72, 49.04, 36.46, 34.80, 33.74, 28.09, 18.25.

General Procedure for Attaching Amino Acid-Anchor-Conjugates 8b−12b to the Resin. *tert*-Butyloxycarbonyl-β-alanyl-(aminomethyl)polystyrene (Boc-β-Ala-PS, 1 g, 1.0−1.2 mmol of β-Ala/g) was shaken with 20 mL of dichloromethane/TFA (1:1) for 45 min. The resin was washed with dichloromethane (6 × 10 mL). After 10 min of shaking with 20 mL of 10% DIEA in dichloromethane, the resin was washed with dichloromethane (6 × 10 mL). Conjugates **8b−12b** were coupled using 3.0 equiv of the carboxylic acid, 3.3 equiv of DIC, and 4.5 equiv of HOBt in the lowest amount of dichloromethane needed for suspending the resin. After 10−15 h of shaking the resin was washed (6 × 10 mL dichloromethane) and treated with 20 mL of pyridine/acetic anhydride (3:1) for 10 min in order to accomplish capping. The resin was washed (6 × 10 mL of dichloromethane) and dried in vacuo.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-alanyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl (Aminomethyl)polystyrene (17a). Starting from 1.62 g (1.57 mmol of β-Ala) of Boc-β-Ala-NH-PS (0.97 mmol of β-Ala/g) and 3.25 g (4.72 mmol)$_{cor}$ of Fmoc-Ala-HYCRON-OH (**8b**), 1.66 g of amino acid-resin was obtained. Loading (AAA): 0.52 mmol of Ala/g, 0.58 mmol of β-Ala/g. Yield: 89%.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)glycyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl (Aminomethyl)polystyrene (18). Starting from 1.61 g (1.93 mmol of β-Ala) of Boc-β-Ala-NH-PS (1.20 mmol of β-Ala/g) and 3.60 g (6.2 mmol)$_{cor}$ of Fmoc-Gly-HYCRON-OH (**9b**), 2.24 g of amino acid-resin was obtained. Loading (AAA): 0.50 mmol of Gly/g, 0.63 mmol of β-Ala/g. Yield: 80%.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-prolyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl (Aminomethyl)polystyrene (19). Starting from 2.20 g (2.64 mmol of β-Ala) of Boc-β-Ala-NH-PS (1.20 mmol β-Ala/g) and 5.53 g (8.5 mmol)$_{cor}$ of Fmoc-Pro-HYCRON-OH (**10b**), 3.11 g of amino acid-resin was obtained. Loading (photometrically by Fmoc-cleavage): 0.52 mmol of Fmoc/g. Yield: 61%.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-valyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl (Aminomethyl)polystyrene (20). Starting from 1.10 g (1.32 mmol β-Ala) of Boc-β-Ala-NH-PS (1.20 mmol β-Ala/g) and 2.47 g (3.9 mmol)$_{cor}$ of Fmoc-Val-HYCRON-OH (**11b**), 1.60 g of amino acid-resin was obtained. Loading (AAA): 0.42 mmol Val/g, 0.56 mmol β-Ala/g. Yield: 75%.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-threonyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl (Aminomethyl)polystyrene (21). Starting from 0.83 g (1.00 mmol β-Ala) of Boc-β-Ala-NH-PS (1.20 mmol β-Ala/g) and 2.04 g (2.9 mmol)$_{cor}$ of Fmoc-Thr(tBu)-HYCRON-OH (**12b**), 1.33 g of amino acid-resin was obtained. Loading (AAA): >0.40 mmol of Thr/g, 0.61 mmol of β-Ala/g. Yield: >66%.

General Procedure for Attaching Amino Acid-Anchor-β-Alanine Conjugates 16a and 16b to the Resin. (Aminomethyl)polystyrene (1 g, 1.55 mmol NH_2/g) was suspended in a solution of 3 equiv of the amino acid-anchor-β-alanine conjugate, 3.3 equiv of DIC, and 4.5 equiv of HOBt in 40 mL of dichloromethane and shaken for 15 h. After washing (dichloromethane, 6 × 15 mL), the resin was shaken in 30 mL of pyridine/acetic anhydride (3:1) for 10 min in order to accomplish capping. The resin was washed (dichloromethane, 7 × 15 mL) and dried in vacuo.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-L-alanyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl(Aminomethyl)polystyrene (17b). Starting from 0.65 g (1.0 mmol NH_2) of (aminomethyl)polystyrene and 2.27 g (3.15 mmol)$_{cor}$ of Fmoc-Ala-HYCRON-β-Ala-OH (**16a**), 1.24 g of amino acid-resin was obtained. Loading (AAA): 0.71 mmol of Ala/g, 0.73 mmol of β-Ala/g. Yield: 89%.

(*E*)-17-(*tert*-Butyloxycarbonyl-L-alanyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl (Aminomethyl)polystyrene (17c). Starting from (aminomethyl)polystyrene (0.73 g, 1.13 mmol NH_2) and 1.82 g (3.28 mmol)$_{cor}$ of Boc-Ala-

HYCRON-β-Ala-OH (**16b**), 1.22 g of amino acid-resin was obtained. Loading (AAA): 0.67 mmol of Ala/g, 0.74 mmol of β-Ala/g. Yield: 75%.

(*E*)-17-Acetoxy-4,7,10,13-tetraoxa-15-heptadecenoic Acid *tert*-Butyl Ester (22). To a solution of cesium acetate (4.50 g, 23.4 mmol) in 250 mL of DMF was added 6.24 g (15.2 mmol) of **4a**. After 3 h of stirring, 1.50 g (7.8 mmol) of cesium acetate was added. The mixture was stirred for additional 2 h. Cesium bromide was separated by filtration over Hyflo, the filtrate was concentrated in vacuo, and the residue was dissolved in diethyl ether. The solution was washed four times with brine and dried over $MgSO_4$. The solvents were removed in vacuo. After careful drying, 5.06 g (85%) of a clear oil was obtained. R_f: 0.36 (petroleum ether/EtOAc, 1:1). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 5.70−5.60 (m, 2H), 4.45 (s, 2H, H-17), 3.92 (s, 2H, H-14), 3.62−3.50 (m, 14H), 2.38 (t, 2H, J = 7.0), 1.95 (s, 3H, Ac), 1.33 (s, 9H). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 170.58, 170.30, 130.78 (C-15), 126.33 (C-16), 80.17, 70.61, 70.44, 70.41, 70.32, 70.17, 69.49, 66.70, 63.96 (C-17), 36.12, 27.89, 20.60 (Ac). Anal. Calcd for $C_{19}H_{34}O_8$: C, 58.44; H, 8.78. Found: C, 57.76; H, 8.95.

(*E*)-17-Acetoxy-4,7,10,13-tetraoxa-15-heptadecenoic Acid (23). A solution of 3.00 g (7.68 mmol) of **22** in 40 mL of TFA was stirred for 45 min. TFA was removed in vacuo and the residue coevaporated with toluene. Chromatography (petroleum ether/EtOAc/AcOH) yielded 2.06 g (90%) of a yellowish oil. R_f: 0.11 (petroleum ether/EtOAc, 1:1). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 9.70 (s, 1H, COOH), 5.77−5.72 (m, 2H), 4.47 (d, 2H, J = 4.1)), 3.95 (d, 2H, J = 3.8), 3.67 (t, 2H, H-3, J = 6.3), 3.56−3.49 (m, 12 H), 2.53 (t, 2H, H-2, J = 6.3), 1.98 (s, 3H). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 175.33 (COOH), 170.63, 130.58, 126.47, 70.58, 70.29, 70.24, 70.10, 69.30, 66.24 (C-3), 64.03, 34.67 (C-2), 20.67. Anal. Calcd for $C_{15}H_{26}O_8$: C, 53.88; H, 7.84. Found: C, 54.01; H, 7.66.

(*E*)-17-Acetoxy-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl (Aminomethyl)polystyrene (24). Boc-removal from 1.11 g (1.67 mmol of β-Ala) of Boc-β-Ala-NH-PS (1.5 mmol of β-Ala/g) and subsequent neutralization was performed as described (see **17a**−**21**). The β-alanyl-resin was suspended in a solution of **23** (1.67 g, 5.00 mmol), HOBt (1.22 g, 7.50 mmol), and DIC (0.85 mL, 5.50 mmol) in 20 mL of dichloromethane, shaken for 16 h, and washed (dichloromethane, 6 × 20 mL). Unreacted amino groups were capped by shaking in pyridine/acetic anhydride (3:1, 20 mL). After washing (dichloromethane, 6 × 20 mL) and drying in vacuo, 1.30 g of **24** was obtained.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-*S*-(acetamidomethyl)-L-cysteinyloxy)-4,7,10,13-tetraoxa-15-heptadecenoyl-β-alanyl (Aminomethyl)polystyrene (25). A solution of 1 N NaOH (34 mL) in 1,4-dioxane (100 mL) was added to 1.30 g of resin **24**. The suspension was shaken for 20 min. After washing, this procedure was repeated once. The resin was washed [2 × 20 mL of dioxane, 2 × 20 mL of dioxane/water (1:1), 2 × 20 mL of water, 2 × 20 mL of DMF, 2 × 20 mL of dichloromethane] and dried, yielding 1.26 g of the dry hydroxyallyl-polymer. A solution of 1.24 g (3.00 mmol) of Fmoc-Cys(Acm)-OH, 0.36 mL (4.50 mmol) of pyridine, and 0.42 mL (3.00 mmol) of 2,6-dichlorobenzoyl chloride in DMF (10 mL) was added to 1.0 g of this resin. After 24 h the resin was washed (6 × 20 mL of DMF) followed by addition of 15 mL of pyridine and 4 mL of benzoyl chloride. The suspension was shaken for 10 min, washed (4 × 20 mL of DMF, 4 × 20 mL of dichloromethane), and dried in vacuo yielding 1.38 g of amino acid-resin. Loading (photometrically by Fmoc-cleavage): 0.59 mmol Fmoc/g.

(*E*)-17-Hydroxy-4,7,10,13-tetraoxa-15-heptadecenoic Acid *tert*-Butyl Ester (26). To a stirred solution of **22** (1.01 g, 2.58 mmol) in 1,4-dioxane (150 mL) was added 0.5 M aqueous NaOH (50 mL). After 20 min the mixture was neutralized with 1 M HCl, and 50 mL of dichloromethane were added. The aqueous layer was extracted three times with dichloromethane. The combined organic layers were washed twice with brine and dried over $MgSO_4$. The solvents were removed in vacuo. After drying, 0.82 g (91%) of a clear and colorless oil were obtained. R_f: 0.16 (petroleum ether/EtOAc, 1:3). 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 5.87−5.79 (m, 2H), 4.13 (s, 2H, H-17), 4.00 (d, 2H, H-14, J = 4.5), 3.68 (t, 2H, J = 6.7), 3.62−3.55 (m, 12H), 2.47 (t, 2H, J = 6.6), 1.42 (s, 9H). 50.3 MHz ^{13}C-NMR ($CDCl_3$) δ = 170.91, 132.50 (C-15), 127.34 (C-16), 80.49, 70.53, 70.41, 70.28, 69.35, 66.81, 62.59 (C-17), 36.20, 28.03. Anal. Calcd for $C_{17}H_{32}O_7$: C, 58.60; H, 9.26. Found: C, 59.05; H, 9.32.

(*E*)-17-(*N*-(9-Fluorenylmethoxycarbonyl)-*S*-(acetamidomethyl)-L-cysteinyloxy)-4,7,10,13-tetraoxa-15-heptadecenoic Acid *tert*-Butyl Ester (27). Pyridine was distilled from **26** (174 mg, 0.50 mmol) for drying. The residue was dissolved in a solution of Fmoc-Cys(Acm)-OH (415 mg, 1.0 mmol) in DMF (4 mL). After addition of pyridine (1.28 mL, 1.50 mmol), 2,6-dichlorobenzoyl chloride (0.14 mL, 1.00 mmol) was added. The solution was stirred for 24 h followed by removal of the solvent in vacuo. The residue was dissolved in dichloromethane and washed with saturated $NaHCO_3$ solution, 1 M HCl, and brine. The aqueous layers were reextracted with dichloromethane. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by chromatography (EtOAc), which furnished 251 mg (68%) of a highly viscous oil. R_f: 0.24 (EtOAc). $[\alpha]^{22}_D$ = −2.7 (c = 1.00, $CHCl_3$). 200 MHz ^{1}H-NMR (DMSO-d_6) δ = 8.52 (t, 1H, NH-Acm, J = 6.7), 7.94−7.87 (m, 3H, H-4-, H-5-Fmoc, C^{NH}), 7.71 (d, 2H, J = 7.2), 7.45−7.26 (m, 4H), 5.82−5.73 (m, 2H), 4.60 (d, 2H, H-17, J = 5.2), 4.31−4.19 (m, 6H, C^{α}, CH_2-, H-9-Fmoc, CH_2-Acm), 3.90 (d, 2H, H-14, J = 4.2), 3.56 (t, 2H, J = 6.3), 3.50−3.31 (m, 12H), 3.00 (dd, 1H, $C^{\beta a}$, $J_{\beta,\beta}$ = 13.7, $J_{\beta,\alpha}$ = 5.0), 2.85 (dd, 1H, $C^{\beta b}$, $J_{\beta,\beta}$ = 13.7), 2.39 (t, 2H, J = 6.2), 1.83 (s, 3H, CH_3-Acm), 1.38 (m, 9H). 50.3 MHz ^{13}C-NMR (DMSO-d_6) δ = 170.54, 170.34, 169.47, 155.92, 143.66, 140.66, 130.67 (C-15), 127.58, 127.00, 125.40 (C-16), 125.16, 120.02, 79.63, 69.69, 68.99, 66.14, 65.78, 64.35 (C-17), 54.04 (C^{α}), 46.53, 39.80 (CH_2-Acm), 35.76, 31.41 (C^{β}), 27.63, 22.45 (CH_3-Acm).

General Procedure for Solid Phase Synthesis of 28−35 and 38. The following amounts of chemicals are based on a synthesis starting from 1 g of the amino acid loaded resin. Boc-removal: 20 mL of dichloromethane/TFA (1:1), 45 min, washing (dichloromethane, 6 × 15 mL). Neutralization: dichloromethane/DIEA (10:1, 15 mL), 10 min, washing (dichloromethane, 6 × 15 mL). Fmoc-removal: Method A: DMF/morpholine (4:3, 25 mL), 2 h 30 min. Method B: DMF/morpholine (1:1, 20 mL), 50 min. Method C: DMF/piperidine (4:3, 20 mL), 10 min; washing (DMF, 6 × 15 mL). Couplings: 3.0−5.0 equiv of Boc- or Fmoc-amino acid, 4.5−7.5 equiv of HOBt in dichloromethane/DMF (10:1) or DMF (resulting concentration 0.12−0.17 M), 3.3−5.5 equiv of DIC (method A) or 3.0−5.0 equiv of TBTU, 6.0−10.0 equiv of NMM (method B), 3−16 h. Washing: dichloromethane or DMF (6 × 15 mL). Incorporation of amino acids with side chain functions was achieved by using amino acid derivatives as follows: Fmoc-Arg(Mtr)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-His(Trt)-OH. The GalNAc-structure was introduced by coupling the preformed glycosyl-amino acid Fmoc-Thr(αAc_3GalNAc)-OH.[34] Capping: pyridine/acetic anhydride (3:1, 15 mL), 10 min, washing (dichloromethane or DMF, 6 × 15 mL).

Boc-Leu-Phe-Ala-OH (28). The synthesis was carried out via Boc-strategy starting from 1.18 g (0.79 mmol Ala) of resin **17c** (0.67 mmol Ala/g, 0.74 mmol β-Ala/g). Boc-amino acids were coupled using DIC. The peptide-resin was dried in vacuo, yielding 1.40 g of the polymer (0.43 mmol Phe/g, 0.45 mmol Ala/g, 0.45 mmol β-Ala/g). The peptide was released by suspending the peptide-resin in a solution of dichloromethane/THF (3:1, 20 mL) and of morpholine (0.7 mL, 8.0 mmol) followed by multiple evacuations and subsequent argon-streamings. After adding tetrakis(triphenylphosphine)palladium (50 mg, 0.04 mmol) the oxygen-free suspension was shaken for 17 h under exclusion of light in an argon atmosphere. Washing (dichloromethane, 6 × 20 mL) and drying in vacuo yielded a polymer with loadings as follows: 0.08 mmol of Leu/g, 0.08 mmol of Phe/g, 0.08 mmol of Ala/g, 0.73 mmol of β-Ala/g (89% detachment yield).

The combined filtrates were washed twice with 30 mL of 0.5 M HCl. The aqueous solutions were extracted with 20 mL of dichloromethane. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification was performed by dissolving the material obtained by chromatog-

raphy (petroleum ether/EtOAc) in ethyl acetate followed by addition of petroleum ether. The colorless solid was collected and dried in vacuo to give 220 mg (63%) of **28**. t_R: 11 min 40 s (column 1, grad 1, 1 mL/min). $[\alpha]^{22}_D = -41.32$ (c = 1.0, MeOH). Mp: 118 °C. 00 MHz ^{1}H-NMR (DMSO-d_6) δ = 8.36 (d, 1H, A^{NH}, J = 7.2), 7.73 (d, 1H, F^{NH}, J = 8.4), 7.22−7.10 (m, 5H, F^{ar}), 6.90 (d, 1H, L^{NH}, J = 8.5), 4.61−4.53 (m,1H, F^{α}), 4.20 (quint, 1H, A^{α}, $J_{\alpha,NH} = J_{\alpha,\beta} = 7.2$), 3.94−3.78 (m, 1H, L^{α}), 3.02 (dd, 1H, $F^{\beta a}$, $J_{\beta,\beta}$ = 13.8, $J_{\beta,\alpha}$ = 3.9), 2.78 (dd, 1H, $F^{\beta b}$, $J_{\beta,\beta}$ = 13.8, $J_{\beta,\alpha}$ = 9.5), 1.44−1.18 (m, 15H), 0.80 (2 × d, 6H, L^{δ}, J = 6.7). 100.6 MHz ^{13}C-NMR ($CDCl_3$) δ = 174.90, 173.06, 170.80, 155.79, 136.15, 129.42, 128.37, 126.80, 80.22, 53.94, 53.30, 48.30, 41.21, 38.20, 28.22, 24.61, 22.74, 21.85, 17.85. Anal. Calcd for $C_{23}H_{35}N_3O_6$: C, 61.45; H, 7.85; N, 9.35. Found: C, 61.34; H, 7.84; N, 9.27.

Fmoc-Leu-Phe-Ala-OH (29). The synthesis was carried out via Fmoc-strategy (method A) starting from 1.64 g (0.81 mmol Ala) of resin **17a** (0.50 mmol Ala/g, 0.69 mmol β-Ala/g). Fmoc-amino acids were activated by DIC. After drying in vacuo 1.40 g of peptide-resin were obtained (0.40 mmol of Leu/g, 0.33 mmol of Phe/g, 0.40 mmol of Ala/g, 0.78 mmol of β-Ala/g). The resin was suspended in dichloromethane/DMSO (1:1, 30 mL) prior to addition of 1.1 mL (10.1 mmol) of *N*-methylaniline. The reaction vessel was evacuated until bubbling of the suspension occurred and then streamed by argon. After five repeats, tetrakis(triphenylphosphine)palladium (100 mg) was added. The mixture was shaken in argon under exclusion of light for 15 h. The resin was washed (DMF, 3 × 20 mL, dichloromethane, 4 × 20 mL) and dried in vacuo, yielding 1.42 g of polymer (0.08 mmol of Leu/g, 0.07 mmol of Phe/g, 0.09 mmol of Ala/g, 0.85 mmol of β-Ala/g, 81% detachment yield). The combined filtrates were concentrated in vacuo. The residue was dissolved in chloroform (50 mL) and washed with 30 mL of 1 M HCl. The aqueous solution was reextracted four times with dichloromethane. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. Two-fold chromatography (1. EtOAc/AcOH, 100:1; 2. chloroform/MeOH) gave 343 mg of peptide, which was dissolved in dichloromethane and precipitated by addition of n-heptane. The colorless solid was collected and dried in vacuo to give 290 mg (62%) of tripeptide. R_f: 0.15 (EtOAc/AcOH, 100:1). $[\alpha]^{22}_D = -39.44$ (c = 1.00, MeOH). Mp: 182 °C. 400 MHz ^{1}H-NMR (DMSO-d_6) δ = 8.27 (d, 1H, NH, J = 7.2), 7.87 (d, 3H), 7.69, 7.68 (2 × d, 2H), 7.46−7.05 (m, 11H), 4.54 (ddd, 1H, F^{α}, $J_{\alpha,\beta a}$ = 4.1, $J_{\alpha,\beta b} \sim J_{\alpha,NH}$ = 8.7), 4.33−4.07 (m, 4H), 3.98−3.92 (m, 1H, L^{α}), 3.02 (dd, 1H, $F^{\beta a}$, $J_{\beta,\beta}$ = 13.9, $J_{\beta,\alpha}$ = 4.0), 2.77 (dd, 1H, $F^{\beta b}$, $J_{\beta,\beta}$ = 13.9, $J_{\beta,\alpha}$ =9.7), 1.50−1.43 (m, 1H, L^{γ}), 1.36−1.29 (m, 2H, L^{β}), 1.27 (d, 3H, A^{β}, J = 7.3), 0.82 (d, 3H, $L^{\delta a}$, J = 6.5), 0.77 (d, 3H, $L^{\delta b}$, J = 6.5). 50.3 MHz ^{13}C-NMR (DMSO-d_6): 173.93, 171.96, 170.67, 155.75, 143.85, 143.62, 140.66, 137.53, 129.25, 127.83, 127.56, 127.00, 125.19, 120.02, 65.48, 53.25, 53.18, 47.54, 46.67, 38.14, 37.47, 24.05, 22.89, 21.42, 17.14. Anal. Calcd for $C_{33}H_{37}N_3O_6$: C, 69.33; H, 6.52; N, 7.35. Found: C, 68.92; H, 6.50; N, 7.17.

Fmoc-Tyr(tBu)-Leu-Ala-OH (30). The synthesis started from 1.21 g (0.84 mmol of Ala) of **17b** (0.71 mmol of Ala/g, 0.73 mmol of β-Ala/g). The Fmoc-strategy was applied (method A). Fmoc-amino acids were coupled using DIC. After drying in vacuo 1.29 g of peptide-resin (0.37 mmol of Tyr/g, 0.42 mmol of Leu/g, 0.46 mmol of Ala/g, 0.70 mmol of β-Ala/g) were obtained. Peptide-release was accomplished as described for the synthesis of **29** using 1.25 g of the peptide-resin, dichloromethane/DMSO (1:1, 30 mL), *N*-methylaniline (1.1 mL, 10.1 mmol), and tetrakis(triphenylphosphine)palladium (200 mg). Drying furnished 0.94 g of polymer (0.03 mmol of Tyr/g, 0.03 mmol of Leu/g, 0.04 mmol of Ala/g, 0.71 mmol of β-Ala/g, 93% detachment yield). After aqueous workup (see synthesis of **29**), the raw material was dissolved in wet MeOH (25 mL) and allowed to stand for 2 h. The palladium was removed by filtration and the filtrate concentrated in vacuo. The residue was purified by chromatography ($CHCl_3$/MeOH), precipitation (MeOH), and then chromatography again (petroleum ether/EtOAc), yielding 330 mg of a yellow foam which was crystallized from EtOAc/*n*-heptane to furnish 270 mg (50%) of a colorless solid. t_R: 15 min 20 s (column 1, grad 1, 1 mL/min. $[\alpha]^{22}_D = -30.5$ (c = 1.0, MeOH). Mp: 163 °C. 200 MHz ^{1}H-NMR ($CDCl_3$) δ = 7.73 (d, 2H), 7.50 (d, 2H), 7.41−7.22 (m, 5H), 7.13 (d, 1H, J = 7.3), 7.00 (d, 2H, $Y^{ar-2,6}$, J = 8.3), 6.83 (d, 2H, $Y^{ar-3,5}$, J = 8.4), 5.60 (d, 1H, NH), 4.58−4.12 (m, 6H, Y^{α}, A^{α}, CH_2-, H-9-Fmoc), 3.02−2.96 (m, 2H, Y^{β}), 1.58−1.40 (m, 6H, L^{γ}, L^{β}, A^{β}), 1.25 (s, 9H), 0.83 (d, 6H, L^{δ}, J = 5.3). 50.3 MHz ^{13}C-NMR (Acetone-d_6) δ = 174.21, 172.80, 172.51, 156.90, 154.99, 144.89, 144.82, 141.92, 133.11, 130.73, 128.41, 127.87, 126.16, 126.09, 124.45, 120.65, 78.21, 67.31, 57.20, 52.19, 48.60, 47.80, 42.18, 38.38, 29.02, 25.13, 23.36, 22.25, 17.83. Anal. Calcd for $C_{37}H_{45}N_3O_7$: C, 69.03; H, 7.05; N, 6.53. Found: C, 69.07; H, 6.97; N, 6.55.

Fmoc-Gly-Leu-Tyr(tBu)-Ala-OH (31). The synthesis was performed applying Fmoc-strategy (method A) and started from 2.31 g (1.44 mmol Ala) of **8b** (0.63 mmol Ala/g, 0.71 mmol β-Ala/g). Fmoc-amino acids were activated by DIC. Drying yielded 2.43 g of peptide-resin (0.35 mmol of Gly/g, 0.32 mmol of Leu/g, 0.28 mmol of Tyr/g, 0.35 mmol of Ala/g, 0.60 mmol of β-Ala/g). Peptide-release was accomplished as described above (see **29**) using 2.39 g of peptide-resin, DMF/DMSO (1:1, 50 mL), *N*-methylaniline (2.0 mL, 18.7 mmol), and 130 mg (0.1 mmol) of palladium(0)-catalyst. After cleavage, 1.85 g of dry polymer (0.02 mmol of Gly/g, 0.02 mmol of Leu/g, 0.00 mmol of Tyr/g, 0.01 mmol of Ala/g, 0.64 mmol of β-Ala/g, 96% detachment yield) was obtained. Aqueous workup (see **29**) and separation of the catalyst by crystallization (see **30**) furnished a brown oil, which was purified by chromatography (EtOAc). The yellowish solid was dissolved in dichloromethane and the colorless solid, which had precipitated upon addition of *n*-heptane, was collected to give 478 mg (47%) of Fmoc-tetrapeptide. t_R: 17 min 30 s (column 1, grad 1, 1 mL/min). $[\alpha]^{22}_D = -24.50$ (c = 1.00, MeOH). Mp: 120−140 °C. 400 MHz ^{1}H-NMR (DMSO-d_6) δ = 8.04 (m, 3H), 7.93 (d, 1H, J = 8.3), 7.88 (d, 2H, J = 7.5), 7.69 (d, 1H, J = 7.4), 7.59 (t, 1H, J = 5.6), 7.40 (t, 2H, J = 7.4), 7.31 (t, 2H, J = 7.4), 7.11 (d, 2H, $Y^{ar-2,6}$, J = 8.2), 6.82 (d, 2H, $Y^{ar-3,5}$, J = 8.2), 4.50−4.46 (m, 1H, Y^{α}), 4.28−4.12 (m, 5H, A^{α}, CH_2-, H-9-Fmoc, L^{α}), 3.64−3.60 (m, 2H, G^{α}), 3.04 (dd, 1H, $Y^{\beta a}$, $J_{\beta,\beta}$ = 13.9, $J_{\beta,\alpha}$ = 3.4), 2.75 (dd, 1H, $Y^{\beta b}$, $J_{\beta,\beta}$ = 13.7, $J_{\beta,\alpha}$ = 10.3), 1.54−1.49 (m, 1H, L^{γ}), 1.32−1.22 (m, 14H, L^{β}, A^{β}, tBu), 0.80, 0.78 (2 × d, 6H, L^{δ}, J = 6.5). 100.6 MHz ^{13}C-NMR (DMSO-d_6) δ = 173.84, 171.59, 170.65, 169.31, 156.57, 153.39, 143.80, 140.71, 132.39, 129.62, 127.60, 127.04, 125.16, 123.26, 120.05, 77.49, 65.84, 53.41, 51.44, 47.62, 46.63, 43.47, 40.71, 36.48, 28.53, 24.04, 22.94, 21.53, 17.08. Anal. Calcd for $C_{39}H_{48}N_4O_8$: C, 66.84; H, 6.90; N, 7.99. Found: C, 66.21; H, 6.99; N, 7.85.

Fmoc-Ala-Ser(tBu)-Thr(αAc$_3$GalNAc)-Thr(tBu)-OH (32). The synthesis was performed via Fmoc-strategy (method B) starting from 1.31 g (0.52 mmol Thr) of **21** (0.40 mmol of Thr/g, 0.61 mmol of β-Ala/g). All amino acids were activated by DIC. After drying, 1.79 g of peptide-resin (0.42 mmol of Ala/g, 0.08 mmol of Ser/g, 0.23 mmol of Thr/g, 0.48 mmol of β-Ala/g) was obtained. Peptide-release was accomplished by treating 1.76 g of peptide-resin with DMF/DMSO (1:1, 30 mL), *N*-methylaniline (1.2 mL, 11.2 mmol), and tetrakis(triphenylphosphine)palladium (100 mg, 0.09 mmol) (see **29**) to give 1.02 g of dried polymer (0.02 mmol of Ala/g, 0.01 mmol of Ser/g, 0.02 mmol of Thr/g, 0.62 mmol of β-Ala/g, 96% detachment yield). Aqueous workup (see **29**) followed by removal of palladium by crystallization (see **30**) yielded 1.1 g of raw material which was purified by two-fold chromatography (EtOAc/AcOH, 50:1). Lyophilization using benzene furnished 630 mg (77%) of a colorless solid. t_R: 8 min 10 s (column 1, grad 1, 1 mL/min). $[\alpha]^{22}_D = +33.53$ (c = 1.0, MeOH). Mp: 120−125 °C. 400 MHz ^{1}H-NMR (^{1}H,^{1}H-COSY, DMSO-d_6) δ = 8.08 (d, 1H, T^{NH}, J = 9.1), 7.90−7.85 (m, 3H, H-4,5-Fmoc, S^{NH}, $J_{4,3} = J_{5,6} = 7.6$, $J_{NH,\alpha}$ = 9.9), 7.79 (d, 1H, T^{NH}, J = 9.1), 7.72 (d, 2H, J = 7.2), 7.75 (d, 1H, NH-Ac, J = 7.8), 7.42 (t, 2H, J = 7.4), 7.33 (t, 2H, J = 7.4), 6.97 (d, 1H, A^{NH}, J = 8.4), 5.29 (d, 1H, H-4′, $J_{4',3'}$ = 3.2), 4.98 (dd, 1H, H-3′, $J_{3',2'}$ = 11.4, $J_{3',4'}$ = 3.2), 4.80 (d, 1H, H-1′, $J_{1',2'}$ = 3.4), 4.76 (dd, 1H, T^{α}, $J_{\alpha,NH}$ = 9.2, $J_{\alpha,\beta}$ = 1.3), 4.57−4.53 (m, 1H, S^{α}), 4.41−4.15 (m, 9H, A^{α}, CH_2-, H-9-Fmoc, T^{α}, T^{β}, H-3′, H-5′), 4.03−3.95 (m, 2H, H-6′), 3.53−3.45 (m, 2H, S^{β}), 2.10, 1.96, 1.87, 1.84 (4 × s, 4 × 3H, 4 × Ac), 1.24 (d, 3H, A^{β}, J = 7.1), 1.16−1.02 (m, 24H, T^{γ}, tBu, J_1 = 6.1, J_2 = 6.2). 100.6 MHz ^{13}C-NMR (DMSO-d_6) δ = 172.45, 171.63, 170.13, 169.89, 169.84, 169.74, 169.65, 169.18, 155.60, 143.77, 143.69, 140.61,

127.52, 126.99, 125.16, 119.98, 98.82 (C-1′), 76.90 (T(Ac_3-GalNAc)$^{\beta}$), 73.16, 72.69, 68.54, 67.27, 66.98, 66.35, 65.63, 61.84, 57.33, 55.37 (T(Ac_3GalNAc)$^{\alpha}$), 52.90, 49.90, 46.59, 46.50, 28.19, 26.95, 22.54, 20.39, 20.35, 20.30, 18.07, 17.89. Anal. Calcd for $C_{51}H_{71}N_5O_{18}$: C, 58.74; H, 6.87; N, 6.72. Found: C, 58.65; H, 6.91; N, 6.71.

Ac-Ala-Pro-Asp(OtBu)-Thr(tBu)-Arg(Mtr)-Pro-Ala-Pro-Gly-OH (33). The synthesis started from 1.28 g (0.49 mmol Gly) of **18** (0.38 mmol of Gly/g, 0.47 mmolof β-Ala/g). After Fmoc-removal (method B), Boc-proline was coupled. Subsequent couplings were performed with Fmoc-amino acids activated by DIC. Couplings were repeated for the N-terminal threonine, aspartic acid, proline and alanine. The terminal alanine was acetylated after Fmoc-cleavage. After drying, 1.85 g of peptide-resin (0.22 mmol of Asp/g, 0.18 mmol of Thr/g, 0.26 mmol of Arg/g, 0.46 mmol of Ala/g, 0.90 mmol of Pro/g, 0.30 mmol of Gly/g, 0.39 mmol of β-Ala/g) were obtained. For peptide-release 1.83 g of peptide-resin were treated with DMSO/DMF (1:1, 20 mL), dichloromethane (4 mL), *N*-methylaniline (1.4 mL, 12.9 mmol), and a small amount of the palladium-catalyst (see **29**). Washing (6 × 15 mL of DMF and dichloromethane) and drying yielded 1.10 g of polymer (0.03 mmol of Asp/g, 0.03 mmol of Thr/g, 0.04 mmol of Arg/g, 0.09 mmol of Ala/g, 0.12 mmol of Pro/g, 0.05 mmol of Gly/g, 0.54 mmol of β-Ala/g, 87% detachment yield). The filtrates were worked up as described (**32**). The raw material was purified by chromatography ($CHCl_3$/MeOH/AcOH, 8:2:0.01). The product fractions were evaporated in vacuo and the residue dissolved in 2-propanol followed by addition of *n*-heptane. The colorless precipitate was collected and dried to give 509 mg (83%) of nonapeptide. t_R: 21 min 30 s (column 1, grad 2, 1 mL/min). $[\alpha]^{22}_D = -92.60$ (c = 1.00, MeOH). Mp: 150−160 °C. 400 MHz ^{1}H-NMR (^{1}H,^{1}H-COSY, ROESY, DMSO-d_6) δ = 8.27 (d, 1H, D^{NH}, J = 7.5), 8.13 (d, 1H, $A_1{}^{NH}$), 8.10−8.06 (m, 2H, G^{NH}, $A_7{}^{NH}$), 7.98 (d, 1H, $R^{\alpha-NH}$, J = 6.0), 7.19 (d, 1H, T^{NH}, J = 6.9), 6.66 (m_c, 2H, H-5-Mtr, $R^{NH(\epsilon,\zeta,\eta)}$), 6.27 (s, 2H, $R^{NH(\epsilon,\zeta,\eta)}$), 4.61−4.50 (m, 4H, D^{α} (4.56), $A_1{}^{\alpha}$ (4.52), R^{α} (4.51), $A_7{}^{\alpha}$ (4.50)), 4.40−4.33 (m, 3H, P^{α}), 4.29−4.22 (m, 1H, T^{α}), 4.01 (m_c, 1H, T^{β}), 3.80 (s, 3H, OCH_3-Mtr), 3.72 (m_c, 2H, G^{α}), 3.60−3.30 (m, P^{δ}, H_2O), 3.03 (m_c, 2H, R^{δ}), 2.70 (m_c, 1H, $D^{\beta a}$), 2.59−2.51 (2 × s, 2 × CH_3-Mtr, DMSO), 2.46 (m_c, 1H, $D^{\beta 2}$), 2.18−1.75 (m, 18H, P^{β}, P^{γ}, Ac, CH_3-Mtr), 1.45−1.39 (m, 13H, R^{β}, R^{γ}, tBu), 1.22−1.16 (m, 6H, $A_1{}^{\beta}$ (1.19) $A_7{}^{\beta}$ (1.16)), 1.08 (s, 9H, tBu), 0.98 (m, 3H, T^{γ}, J = 6.3). 100.6 MHz ^{13}C-NMR (MeCN-d_3) δ = 173.37, 173.29, 173.08, 172.84, 172.61, 172.35, 172.02, 171.73, 171.37, 170.87, 170.06, 159.25, 157.76, 139.27, 137.38, 135.38, 125.39, 112.92, 82.02, 75.53, 67.59, 61.41, 61.30, 58.89, 56.32, 51.67, 50.98, 49.16, 48.28, 22.28, 47.54, 48.01, 41.82, 37.71, 32.20, 29.89, 29.67, 29.43, 28.67, 28.34, 25.77, 25.69, 25.23, 24.26, 22.87, 18.76, 18.50, 18.01, 17.74, 12.28. HRMS (FAB, nba, neg): 1248.1 (M(2 × ^{13}C)-H^+, 6.81%, 1247.7 calcd), 1247.1 (M(1 × ^{13}C) − H^+, 21.40%, 1246.7 calcd), 1246.0 (M − H^+, 32.25%, 1245.7 calcd).

Ac-Ala-Pro-Asp(OtBu)-Thr(αAc$_3$GalNAc)-Arg(Mtr)-Pro-Ala-Pro-Gly-OH (34). The synthesis started from 0.85 g (0.42 mmol of Gly) of **18** (0.50 mmol of Gly/g, 0.63 mmol of β-Ala/g). After Fmoc-removal (method B) and coupling of Boc-proline the following couplings were performed with Fmoc-amino acids. Fmoc-Thr(αAc$_3$GalNAc)-OH and Fmoc-Asp(OtBu)-OH were activated by TBTU. All other amino acids were activated by DIC. Couplings were repeated for the N-terminal proline and alanine. The terminal alanine was acetylated after Fmoc-cleavage. Drying yielded 1.49 g of peptide-resin (0.30 mmol of Asp/g, 0.24 mmol of Thr/g, 0.32 mmol of Arg/g, 0.58 mmol of Ala/g, 0.90 mmol of Pro/g, 0.35 mmol of Gly/g, 0.40 mmol of β-Ala/g). The peptide-release was accomplished using 1.47 g of resin, DMSO/DMF (1:1, 16 mL), dichloromethane (2 mL), morpholine (1.2 mL, 13.7 mmol), and a small amount palladium-catalyst (see **29**). After washing and drying, 0.74 g of polymer (0.03 mmol of Asp/g, 0.02 mmol of Thr/g, 0.03 mmol of Arg/g, 0.06 mmol of Ala/g, 0.09 mmol of Pro/g, 0.04 mmol of Gly/g, 0.66 mmol of β-Ala/g, 93% detachment yield) was obtained. The combined filtrates were concentrated in vacuo and coevaporated with DMF. The residue was dissolved in dichloromethane followed by two-fold washing with 0.5 M HCl. After reextraction of the aqueous layers the combined organic layers were washed with brine, which again was reextracted. The combined organic layers were evaporated in vacuo and the residue dissolved in wet methanol. The solution was kept at 0 °C overnight and the yellow solid, which had precipitated, was removed by filtration. The filtrate was concentrated in vacuo and dissolved in dichloromethane. The precipitate was separated by filtration. After evaporation of the filtrate the residue was purified by three-fold chromatography ($CHCl_3$/MeOH/AcOH). The material was dissolved in 2-propanol. Benzene was added, and the solution was subjected to lyophilization to furnish 605 mg (95%) of a colorless solid. t_R: 24 min 10 s (column 2, grad 3, 2 mL/min). $[\alpha]^{22}_D = -20.50$ (c = 1.02, DMSO). Mp: 146−152 °C. 400 MHz ^{1}H-NMR (^{1}H, ^{1}H-COSY, ^{1}H, ^{13}C-COSY, DMSO-d_6) δ = 8.27 (d, 1H, $R^{\alpha-NH}$, J = 6.6), 8.12, 8.10 (2 × d, 2H, D^{NH}, A^{NH} or T^{NH}, J_1 = 7.0, J_2 = 6.1), 8.03 (t, 1H, G^{NH}, J = 5.8), 7.97 (d, 1H, A^{NH} or T^{NH}, J = 7.1), 7.69 (d, 1H, A^{NH}, J = 9.0), 7.05 (d, 1H, GalNAc^{NH}, J = 9.5), 6.67 (m_c, 2H, H-5-Mtr, $R^{NH(\epsilon,\zeta,\eta)}$), 6.42 (s, 2H, $R^{NH(\epsilon,\zeta,\eta)}$), 5.27 (s, 1H, H-4′), 4.91 (dd, 1H, H-3′, $J_{3',2'}$ = 11.5, $J_{3',4'}$ = 3.1), 4.77 (d, 1H, H-1′, $J_{1',2'}$ = 3.4), 4.64 (m, 1H, D^{α}), 4.50−4.41 (m, 3H, A^{α}, T^{α}), 4.37−4.28 (m, 4H, R^{α} (4.37), 3 × P^{α}), 4.21−4.14 (m, 3H, T^{β}, H-2′, H-5′), 4.01 (m_c, 2H, H-6′), 3.77 (s, 3H, OCH_3-Mtr), 3.71−3.63 (m, 2H, G^{α}), 3.61−3.35 (m, P^{δ}, H_2O), 3.01 (m_c, 2H, R^{δ}), 2.70 (dd, 1H, $D^{\beta a}$, $J_{\beta,\beta}$ = 16.0, $J_{\beta,\alpha}$ = 6.1), 2.58 (s, 3H, CH_3-Mtr), 2.50−2.46 (m, CH_3-Mtr, DMSO, $D^{\beta b}$), 2.10−1.64 (m, 30H, P^{β}, P^{γ}, Ac, CH_3-Mtr), 1.43−1.22 (m, 13H, R^{β}, R^{γ}, tBu), 1.18−1.15 (2 × d, 6H, 2 × A^{β}, J_1 = 6.2, J_2 = 6.1), 1.09 (d, 3H, T^{γ}, J = 6.2). 100.6 MHz ^{13}C-NMR (^{1}H,^{13}C-COSY, DMSO-d_6) δ = 171.73, 171.58, 171.00, 170.96, 170.80, 170,45, 170.40, 169.90, 169.81, 169.70, 169.26, 169.26, 169.19, 169.12, 168.83, 157.41, 156.12, 137.50, 135.52, 134.61, 123.44, 111.67, 98.15 (C-1′), 80.17 (tBu), 75.42 (T^{β}), 68.50 (C-3′), 67.17 (C-4′), 66.23 (C-5′), 61.90 (C-6′), [59.30, 59.07 (P^{α})], 55.53 (T^{α}), 55.39, 50.12 (R^{α}), 49.42 (D^{α}), [46.55, 46.43 (P^{δ}, A^{α}, C-2′)], 40.56 (G^{α}), 39.70 (R^{δ}), 36.98 (D^{β}), [28.91, 28.46, 28.11 (R^{β}, P^{β})], 27.52 (tBu), [24.92, 24.22 (R^{γ}, P^{γ})], 23.39, 22.64, 22.12, 20.88, 20.32, 18.14 (T^{γ}), 17.83, 16.76 (2 × A^{β}), 11.59. HRMS (FAB, glyc, pos): 1521.6 (M(1 × ^{13}C) + H^+, 0.88%, 1521.7 calcd), 1520.9 (M + H^+, 1.25%, 1520.7 calcd), 1309.8 (M(1 × ^{13}C) − Mtr + H + H^+, 1.41%, 1309.6 calcd), 1308.7 (M − Mtr + H + H^+, 1.94%, 1308.6 calcd), 213.0 (Mtr^+, 2.12%, 213.1).

Fmoc-Gly-Ser(tBu)-Thr(tBu)-Ala-Pro-Pro-Ala-His(Trt)-Gly-Val-Thr(tBu)-Ser(tBu)-Ala-Pro-Asp(tBu)-Thr(αAc$_3$-GalNAc)-Arg(Mtr)-Pro-Ala-Pro-OH (35). The synthesis started from 1.07 g (0.55 mmol of Fmoc) of **19** (0.52 mmol of Fmoc/g). After Fmoc-removal (method B), Boc-alanine was coupled. The subsequent couplings were performed with Fmoc-amino acids, which were activated by TBTU. After drying, 2.29 g of peptide resin (0.17 mmol of Fmoc/g) was obtained. The peptide was released by adding DMSO/DMF (1:1, 12 mL), *N*-methylaniline (1.0 mL, 9.2 mmol), and a small amount of palladium-catalyst to 1.81 g of the peptide-resin (see **29**). Washing and drying yielded 0.73 g of polymer (0.02 mmol of Fmoc/g, 95% detachment yield). Workup was performed as described above. The HCl-washing was substituted by washing with 0.5 M citric acid. Palladium was removed by crystallization from methanol (see **32**) to give a yellow amorphous solid. Purification was achieved by chromatography ($CHCl_3$/MeOH/AcOH) followed by GPC (Sephadex LH20, $CHCl_3$/MeOH, 1:1). Lyophilization from benzene/2-propanol (10:1) yielded 611 mg (45%) of a colorless solid. t_R: 21 min 10 s (column 3, grad 4, 1 mL/min). $[\alpha]^{22}_D$ −43.07 (c = 0.76, MeOH). HRMS (FAB, nba, pos): 3212.0 ($M_{average}$ + K^+, 3212.7 calcd), 3176.9 (M(4 × ^{13}C) + H^+, 0.03%, 3176.6 calcd), 3175.7 (M(3 × ^{13}C) + H^+, 0.05%, 3175.6 calcd), 3174.6 (M(2 × ^{13}C) + H^+, 0.06%, 3174.6 calcd), 3173.8 (M(1 × ^{13}C) + H^+, 0.04%, 3173.6 calcd), 3173.1 (M + H^+, 0.04%, 3172.6 calcd).

Ac-Ala-Pro-Asp-Thr(αAc$_3$GalNAc)-Arg-Pro-Ala-Pro-Gly-OH·AcOH (36). To 51 mg (33.5 μmol) of glycononapeptide **35** were added anisole (0.06 mL) and ethyl methyl sulfide (0.06 mL). After 6 h of stirring the solution was cooled to 0 °C for 17 h. The solution was concentrated in vacuo, and the residue was dissolved in methanol. The hydrotrifluoroacetate precipitated upon addition of dry diethyl ether. The colorless solid was collected, washed with diethyl ether, and dried in vacuo. The fitrate was concentrated in vacuo and the residue dis-

solved in methanol followed by addition of diethyl ether. The precipitate was collected and washed with diethyl ether. The precipitates were combined and purified by GPC (Sephadex G15, 0.1 M aqueous NH_4OAc, 0.8 mL/min). Lyophilization yielded 41 mg (93%) of glycopeptide **36**. t_R: 22 min 0 s (column 4, grad 5, 1 mL/min). 400 MHz ^{1}H-NMR (D_2O) δ = 5.42 (d, 1H, J = 2.6), 5.17 (dd, 1H, J_1 = 11.2, J_2 = 2.9), 5.02 (d, 1H, J = 3.6), 4.88−4.70 (m, D$^\alpha$, HDO), 4.59−4.32 (m, 10H), 4.23−4.4.20 (m, 2H), 3.82−3.61 (m, 8H), 3.22 (m$_c$, 2H), 2.77 (dd, 1H, J_1 = 16.o, J_2 = 7.0), 2.62 (dd, 1H, J_1 = 16.0, J_2 = 6.0), 2.30−2.21 (m, 6H), 2.08−1.85 (m, 24H), 1.80−1.67 (m, 4H), 1.38, 1.35 (2 × d, 6H, J_1 = J_2 = 7.1), 1.26 (d, 3H, J = 6.3). 100.6 MHz ^{13}C-NMR (D_2O) δ = 173.85, 173.74, 173.68, 173.59, 173.44, 173.30, 173.06, 171.37, 171.14, 156.94, 98.67, 76.22, 69.25, 68.19, 67.05, 62.63, 60.73, 60.42, 60.18, 57.00, 51.44, 51.29, 47.74, 47.62, 40.61, 29.38, 29.28, 27.39, 24.68, 24.63, 24.57, 24.17, 22.15, 21,55, 20.16, 20.09, 17.99, 15.64, 15.52. HRMS (FAB, glyc, pos): 1254.8 (M(2 × ^{13}C) + H$^+$, 2.46%, 1254.6 calcd), 1253.9 (M(1 × ^{13}C) + H$^+$, 5.72%, 1253.6 calcd), 1252.9 (M + H$^+$, 10.90%, 1252.6 calcd).

Ac-Ala-Pro-Asp-Thr(αGalNAc)-Arg-Pro-Ala-Pro-Gly-OH·AcOH (37). A solution of 79 mg (60.2 μmol) of **36** in 10 mL of dry methanol was adjusted to pH 8.5 by addition of a 0.014 M solution of sodium methanolate in methanol. After 1.5 h of stirring, the solution was acidified to pH 6 by addition of acetic acid. The solution was concentrated in vacuo. The residue was dissolved in water and lyophilized. Purification by GPC (Sephadex G15, 0.1 M NH_4OAc, 0.8 mL/min) and lyophilization yielded 59 mg (83%) of glycopeptide. t_R: 17 min 0 s (column 4, grad 6, 1 mL/min). $[\alpha]^{22}_D$ = −112.49 (c = 1.00, H_2O). Mp: 205−210 °C. 400 MHz ^{1}H-NMR (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-COSY, H_2O) δ = 8.32 (d, 1H, J = 6.4), 8.25−8.23 (m, 2H), 8.15 (d, 1H, J = 7.2), 8.05 (d, 1H, J = 5.6), 7.89 (s, 1H, G^{NH}), 7.60 (d, 1H, GalNAc^{NH}, J = 9.6), 7.14 (m, 1H, R$^{\epsilon NH}$), 6.53 (s, 2H, R^{NH}), 5.0−4.6 (m, H-1′, D$^\alpha$, H_2O), 4.59−4.20 (m, 8H, 2 × A$^\alpha$, R$^\alpha$ (4.50), P$^\alpha$, T$^\alpha$ (4.33), T$^\beta$ (4.27)), 4.05 (m$_c$, 1H, H-2′), 3.96−3.90 (m, 2H, H-5′ (3.96), H-4′ (3.92)), 3.85−3.45 (m, 11H, H-3′ (3.83), H-6′ (3.59), G$^\alpha$ (3.75), P$^\delta$ (3.79−3.54)), 3.17 (m$_c$, 2H, R$^\delta$), 2.82−2.61 (m, 2H, D$^\beta$), 2.25 (m$_c$, 3H, P$^{\beta a}$), 2.06−1.73 (m, 15H, P$^{\beta b}$, P$^\gamma$, Ac), 1.71−1.60 (m, 4H, R$^\beta$, R$^\gamma$), 1.22, 1.19 (2 × d, 6H, 2 × A$^\beta$, J_1 = 7.2, J_2 = 6.8), 1.09 (d, 3H, T$^\gamma$, J = 6.4). 100.6 MHz ^{13}C-NMR (^{1}H,^{13}C-COSY, DEPT, D_2O) δ = 173.97, 173.88, 173.75, 173.45, 171.25, 171.07, 156.89, 98.60 (C-1′), 75.24 (T$^\beta$), 71.45 (C-5′), 68.65 (C-4′), 68.12 (C-3′), 61.31 (C-6′), [60.66, 60.46, 60.12 (P$^\alpha$)], 57.19 (T$^\alpha$), 51.29 (R$^\alpha$), 50.85 (D$^\alpha$), 49.77 (C-2′), 47.71 (P$^\delta$, A$^\alpha$, G$^\alpha$), 40.60 (R$^\delta$), 31.25 (D$^\beta$), 29.36 (P$^\beta$), 27.53 (R$^\beta$), [24.64, 24.56 (P$^\gamma$)], 24.16 (R$^\gamma$), 22.36, 21.50 (Ac), 18.33 (T$^\gamma$,), [15.56, 15.44 (A$^\beta$)]. HRMS (FAB, glyc, pos): 1128.7 (M(2 × ^{13}C) + H$^+$, 2.44%, 1128.5 calcd), 1127.7 (M(1 × ^{13}C) + H$^+$, 6.34%, 1127.5 calcd), 1126.8 (M + H$^+$, 10.67%, 1126.5 calcd).

Fmoc-Thr-Leu-Val-OH (38). The synthesis was performed via Fmoc-strategy (method C) and started from 1.56 g (0.65 mmol Val) of **20** (0.42 mmol of Val/g, 0.56 mmol of β-Ala/g). The Fmoc-Thr(tBu)-Leu-Val-resin (0.30 mmol of Thr/g, 0.44 mmol of Leu/g, 0.48 mmol ofVal/g, 0.53 mmol of β-Ala/g) was treated with 20 mL of TFA for 2 h. After washing with DMF (5 × 15 mL) and dichloromethane (4 × 15 mL) and drying, 1.65 g of tripeptide-resin (0.30 mmol of Thr/g, 0.43 mmol of Leu/g, 0.44 mmol of Val/g, 0.55 mmol of β-Ala/g) with unprotected hydroxyl group was obtained. The peptide was released by treating 0.73 g (≈0.32 mmol of peptide) of peptide-resin with DMF/DMSO (1:1, 8 mL), *N*-methylaniline (0.9 mL, 7.1 mmol), and a small amount of palladium(0)-catalyst (see **29**). Washing (6 × 15 mL of DMF, 5 × 15 mL of dichloromethane) and drying yielded 0.60 g of polymer (0.09 mmol of Thr/g, 0.11 mmol of Leu/g, 0.12 mmol of Val/g, 0.91 mmol of β-Ala/g, 84% detachment yield). The filtrates were worked-up as described (see **32**). Purification was achieved by two-fold chromatography (1. $CHCl_3$/MeOH/AcOH, 2. EtOAc/AcOH. 100:1) yielding 170 mg (96%) of a colorless solid. t_R: 24 min 0 s (column 2, grad 7, 1 mL/min). $[\alpha]^{22}_D$ = −36.39 (c = 1.00, MeOH). Mp: 92 °C. 400 MHz ^{1}H-NMR (DMSO-d_6) δ = 7.94−7.87 (m, 4H, H-4-, H-5-Fmoc, V^{NH}, L^{NH}), 7.73 (m$_c$, 2H,), 7.40 (t, 2H, J = 7.4), 7.31 (t, 2H, J = 7.4), 7.17 (d, 1H, T^{NH}, J = 8.7), 4.83 (s, 1H, T^{OH}), 4.45−4.39 (m, 1H), 4.29−4.19 (m, 3H), 4.09−4.06 (m, 1H), 4.00−3.96 (m, 1H), 3.92−3.86 (m, 1H, L$^\alpha$), 2.04−1.99 (m, 1H, V$^\beta$), 1.65−1.59 (m, 1H, L$^\gamma$), 1.47−1.44 (m, 2H, L$^\beta$), 1.03 (d, 3H, T$^\gamma$, J = 6.2), 0.86−0.81 (m, 12H, L$^\delta$, V$^\gamma$). 100.6 MHz ^{13}C-NMR (DMSO-d_6) δ = 172.57, 171.97, 169.78, 155.94, 143.80, 143.66, 140.62, 127.53, 126.97, 125.18, 119.97, 66.71 (T$^\beta$), 65.71, 60.37 (T$^\alpha$), 57.21 (V$^\alpha$), 50.84 (L$^\alpha$), 46.62, 40.74 (L$^\beta$), 29.70 (V$^\beta$), 23.95 (L$^\gamma$), 23.00, 21.57, [9.49, 18.91, 17.97 (L$^\delta$, V$^\gamma$, T$^\gamma$)]. Anal. Calcd for $C_{30}H_{39}N_3O_7$: C, 65.08; H, 7.10; N, 7.59. Found: C, 64.43; H, 6.94; N, 7.44.

Fmoc-Thr(βAc$_4$Glc)-Leu-Val-OH (39). The Fmoc-TLV-resin (0.61 g, ≈0.27 mmol of peptide), which had been dried carefully in vacuo over P_4O_{10}, was washed intensively with dry dichloromethane. The resin was suspended in 30 mL of dry dichloromethane followed by addition of 800 mg (1.85 mmol) of 1-*O*-(*N*-allylcarbamoyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose.[41] After addition of 290 mg (0.61 mmol) of *S*-methylbis(methylthio)sulfonium hexachloroantimonate, the suspension was shaken for 30 min. The resin was washed (dry dichloromethane, 3 × 10 mL) and the glycosylation reaction was repeated twice: 1. with 800 mg (1.85 mmol) of donor and 290 mg (0.61 mmol) of promotor, 2. with 800 mg donor and 270 (0.57 mmol) of promotor. The resin was washed (dichloromethane, 3 × 10 mL; DMF, 6 × 10 mL; pyridine, 4 × 10 mL; DMF, 7 × 10 mL; dichloromethane, 4 × 10 mL) and dried to give 0.70 g of resin (0.35 mmol of Thr/g, 0.51 mmol of Leu/g, 0.56 mmol of Val/g, 0.73 mmol of β-Ala/g). The peptide-release was accomplished using 0.68 g of resin, DMSO/DMF (1:1, 10 mL), dichloromethane (1 mL), *N*-methylaniline (0.9 mL, 8.3 mmol), and a small amount of palladium(0)-catalyst (see **29**). After washing (13 × 15 mL of DMF, 4 × 15 mL of pyridine, 4 × 15 mL of dichloromethane) the cleavage-reaction was repeated twice. Intensive washing and drying in vacuo furnished 0.53 g of polymer (0.16 mmol of Thr/g, 0.21 mmol of Leu/g, 0.22 mmol of Val/g, 0.71 mmol of β-Ala/g, 59% detachment yield). The combined filtrates were worked up as described above (see **32**). Two-fold chromatography (EtOAc/AcOH, 100:1) gave glycopeptide **39** and unglycosylated peptide **38**. The product fractions were evaporated in vacuo and lyophilized from benzene/2-propanol (10:1), yielding 96 mg (64% based on peptide load) of unglycosylated peptide **38** and 10 mg (4.2% based on peptide load) of glycopeptide **39**. t_R: 31 m 20 s (column 2, grad 7, 1 mL/min). $[\alpha]^{22}_D$ = 0.33 (c = 1.91, MeOH). Mp: 84 °C. 400 MHz ^{1}H-NMR (^{1}H,^{1}H-COSY, DMSO-d_6) δ = 7.91−7.87 (m, 3H, H-4-, H-5-Fmoc, V^{NH}), 7.81 (d, 1H, L^{NH}, J = 7.8), 7.69 (m$_c$, 2H), 7.40 (t, 2H, J = 7.4), 7.31 (t, 2H, J = 7.5), 6.97 (d, 1H, T^{NH}, J = 7.9), 5.23 (dd, 1H, H-3′, $J_{3',2'}$ = $J_{3',4'}$ = 9.6), 4.90−4.84 (m, 2H, H-1′, H-4′), 4.70 (dd, 1H, H-2′, $J_{2',1'}$ = $J_{2',3'}$ = 8.8), 4.38 (m$_c$, 1H, L$^\alpha$), 4.24−4.14 (m, 4H, CH_2-Fmoc, H-6′), 4.11−4.05 (m, 2H, V$^\alpha$, T$^\alpha$), 3.99−3.91 (m, 3H, H-9-Fmoc, T$^\beta$, H-5′), 2.03−1.89 (m, 13H, Ac, V$^\beta$), 1.63−1.59 (m, 1H, L$^\gamma$), 1.43−1.37 (m, 2H, L$^\beta$), 1.07 (d, 3H, T$^\gamma$, J = 6.2), 0.86−0.80 (m, 12H, L$^\delta$, V$^\gamma$). 100.6 MHz ^{13}C-NMR (DEPT, DMSO-d_6 (signal doubling in DEPT-spectra, no doubling in 1D-spectrum)) δ = 172.6, 171.60, 169.8, 169.1, 169.0, 168.8, 168.5, 143.7, 140.59, 128.89, 127.61, 127.25, 127.02, 125.19, 121.33 (?), 120.06, 119.98, 98.10, 97.84 (C-1′), [77.75, 75.66, 71.98, 71.91, 70.91, 70.48, 68.19 (C-2′, −3′, −4′, −5′, T$^\beta$)], 65.79, 61.81, 61.70 (C-6), 58.69 (T$^\alpha$), 57.22, 50.95, 50.53, 46.53, 41.17, 40.78, 29.76, 23.87, 23.07, 22.99, 21.71, 21.61, 20.39, 20.28, 20.20, 18.96, 17.98, 16.41, 16.33. HRMS (FAB, nba, pos): 885.9 (M(1 × ^{13}C) + H$^+$, 1.42%, 885.4 calcd), 884.9 (M + H$^+$, 3.26%, 884.4 calcd).

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Supporting Information Available: ^{1}H-NMR spectra of **3**, **4a**, **6**, **8a**−**12a**, **8b**−**12b**, **13**−**15**, **16a/b**, **27**, **33**−**37** and **39**; ^{1}H,^{1}H-COSY of **33** and **34**; ^{1}H,^{13}C-COSY of **37**; RP-HPLC of **35** (29 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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