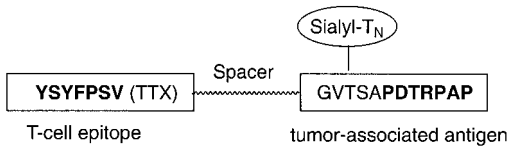
[29] The ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **1** were remeasured in $CDCl_3$. For the ^{1}H NMR shift of **1**, see also M. Jonathan, S. Gordon, B. P. Dailey, *J. Chem. Phys.* **1962**, *36*, 2443 – 2448.
[30] P. W. Fowler, E. Steiner, R. Zanasi, B. Cadioli, *Mol. Phys.* **1999**, *96*, 1099 – 1108.
[31] J. M. Schulman, R. L. Disch, *J. Comput. Chem.* **1998**, *19*, 189 – 194.
[32] L. T. Scott, P.-C. Cheng, M. M. Hashemi, M. S. Bracher, D. T. Meyer, H. B. Warren, *J. Am. Chem. Soc.* **1997**, *119*, 10963 – 10968, and references therein.
[33] A. Ayalon, M. Rabinovitz, P.-C. Cheng, L. T. Scott, *Angew. Chem.* **1992**, *104*, 169 – 170; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1636 – 1637.
[34] A. Pasquarello, M. Schlüter, R. C. Haddon, *Phys. Rev. A* **1993**, *47*, 1783 – 1789.
[35] P. Lazzeretti, R. Zanasi, SYSMO package (University of Modena), **1980**. Some additional routines for the evaluation and plotting of current density were written in Exeter (E. Steiner, P. W. Fowler, unpublished results).
[36] R. D. Amos, J. E. Rice, *The Cambridge Analytical Derivatives Package*, *Issue 4.0*, **1987**.
[37] J. C. Hanson, C. E. Nordman, *Acta Crystallogr. Sect. B* **1976**, *32*, 1147 – 1153.

Towards the Development of Antitumor Vaccines: A Synthetic Conjugate of a Tumor-Associated MUC1 Glycopeptide Antigen and a Tetanus Toxin Epitope**

Stefanie Keil, Christine Claus, Wolfgang Dippold, and Horst Kunz*

Since it was recognized that tumor cells and normal cells are distinctly different in the glycoprotein profile of their outer cell membranes,[1] numerous efforts have been made to develop tumor-selective antigens for vaccinations against tumors.[2] First investigations focused on glycolipids.[3] Since 1977, Springer et al.[1b] have described glycoproteins carrying the Thomsen – Friedenreich antigen (T- or TF-antigen: Galβ(1,3)-GalNAc-O-Ser/Thr) as being tumor-associated surface structures on epithelial cells. A close structural relation between T-antigen glycoproteins and asialoglycophorins was supposed because of cross reactivities of antibodies induced by these components. Based on these results, we synthesized T_N- and T-antigen glycopeptides 15 years ago in order to obtain antigens for vaccinations against tumors.[4] To this end, synthetic T-antigen glycopeptides with the N-terminal sequence of the M blood group glycophorin were coupled to bovine serum albumin (BSA) and used for vaccination of mice. A monoclonal antibody (82-A6) obtained by hybridoma techniques was selectively directed against the T-antigen glycopeptide. It exhibited affinity to epithelial tumor cells in enzyme-linked immunosorbant assays (ELISAs), but did not show sufficient tumor selectivity. Further characterizations of this antibody disclosed no affinity for glycophorin, but binding to asialoglycophorins was observed,[5, 6] which confirms specificity towards the T-antigen structure. Interestingly, the antibody proved to be distinctly more reactive to asialoglycophorins of the M blood group than those of the N blood group. The former contains an N-terminal peptide sequence identical to the one in the synthetic T-antigen glycopeptide. Thus, the antibody differentiated between the two glycoproteins which both carry numerous identical T-antigen saccharides but are slightly different in their peptide sequences. This observation implies that the specificity of an antibody directed against a carbohydrate epitope is distinctly modulated by the peptide sequence in the linkage region.[4–6] We conclude that a tumor-selectitve antigen not only requires a tumor-associated saccharide epitope but also a tumor-selective peptide structure.

During recent years, amino acid sequences of tumor-associated mucins have been elucidated.[7] Our syntheses are now aimed at glycopeptides of the tumor-associated mucin MUC1.[8] MUC1 is overexpressed up to tenfold on epithelial tumors and is incompletely glycosylated because of a prematurely occurring sialylation. The low glycosylation rate of the polymorphic epithelial mucin (PEM) MUC1 influences its conformation. Peptide epitopes located in its tandem repeat region become accessible to the immune system. They should constitute tumor-selective peptide-structure information. Therefore, we have synthesized T_N-,[9] T-,[10] and sialyl – T_N – antigen glycopeptides[11] from the tandem repeat region of MUC1.[12] As only one of these glycopeptides exhibited a proliferating effect on peripheric blood lymphocytes, we have developed a novel concept for the antigen construction of antitumor vaccines. According to this strategy, the tumor-associated MUC1 glycopeptide antigen is combined with a T-cell epitope of tetanus toxin using a flexible spacer to give a conjugate **1** (Figure 1).



1

Figure 1.

The peptide motif PDTRPAP was incorporated into the target structure as the immunodominant domain[13]. The neuraminic acid was protected as its benzyl ester,[14] and the whole construct **1** was formed from two large portions in a solid-phase fragment condensation. The sialyl – T_N building block was synthesized from a T_N – threonine conjugate and a neuraminic acid donor.[11] The O-acetylated sialic acid benzyl ester **2**[14] was treated with acetyl chloride to give the glycosyl chloride which, with potassium *O*-ethylxanthogenate, was

[*] Prof. Dr. H. Kunz, Dipl.-Chem. S. Keil
Institut für Organische Chemie der Universität Mainz
Duesbergweg 10 – 14, 55099 Mainz (Germany)
Fax: (+49) 6131-392-4786
E-mail: hokunz@mail.uni-mainz.de
Dr. C. Claus, Prof. Dr. W. Dippold[+]
Naturwissenschaftlich-Medizinisches Forschungszentrum
der Universität Mainz
[+] Additional address: St. Vincenz-Hospital Mainz

[**] This work was supported by the Deutsche Forschungsgemeinschaft and by the Stiftung Rheinland-Pfalz für Innovation. S.K. is grateful for a Kekulé-Stipendium from the Fonds der Chemischen Industrie.

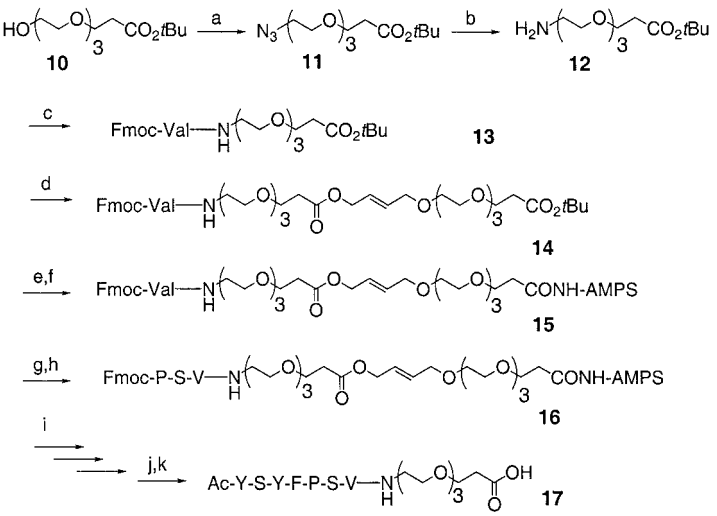
  1433-7851/01/4002-0366 $ 17.50+.50/0

converted into the sialyl xanthogenate **3**;[15] this had already been demonstrated as a favorable glycosyl donor in the case of the sialic acid methyl ester.[11] Reaction of **3** with the partially protected T_N–threonine precursor **4**[11] regioselectively furnished the desired ST_N–threonine conjugate **5** (ST_N = Sialyl-T_N). Low temperature and intermediate acetonitrile conjugates favor the formation of the α-sialoside (Scheme 1).

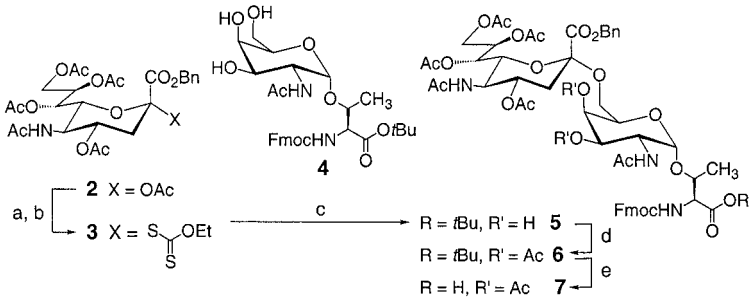
After O-acetylation of **5**, the *tert*-butyl ester was removed from **6** by careful acidolysis. The obtained ST_N–threonine building block **7**[16] was used in the solid-phase synthesis of the glycododecapeptide sequence of the MUC1 tandem repeat region.

The synthesis of the ST_N–glycododecapeptide started with Fmoc-proline linked to the aminomethylpolystyrene (AMPS) resin **8** through the allylic HYCRON anchor[9] and β-alanine. In order to avoid the formation of a diketopiperazine, a change to the Boc strategy was chosen for the second amino acid (Ala). This is possible because of the stability of the allylic anchor to both acids and bases.[9] The further synthesis of the resin-bound ST_N–glycododecapeptide **9** was performed according to the Fmoc strategy (Scheme 2).

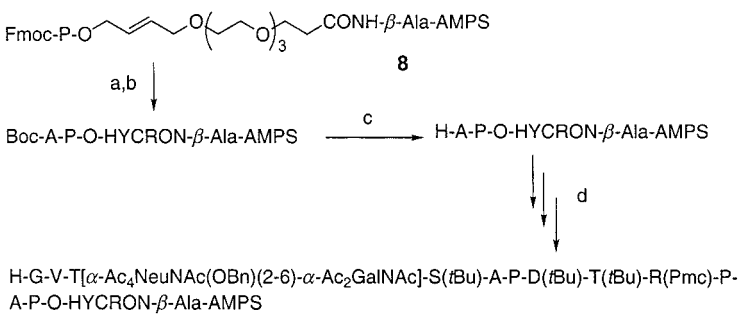
Parallel to this, *tert*-butyl 12-hydroxy-4,7,10-trioxa-dodecanoate[17] (**10**) was converted through the 12-azido derivative **11** into the 12-amino derivative **12**, which was then condensed with Fmoc-valine to give **13**; after cleavage of the *tert*-butyl ester, **13** was coupled to the HYCRON linker.[9] The Fmoc-Val–spacer–HYCRON conjugate **14** was coupled to the polymer (→**15**) and then the tetanus toxin peptide–spacer conjugate was assembled according to the Fmoc strategy. In the first chain extension, a dipeptide was coupled[18] to furnish **16** in order to avoid formation of a diketopiperazine. Finally, the Fmoc group was exchanged for an acetyl group (Scheme 3).



Scheme 3. a) 1. MesCl, NEt_3, CH_2Cl_2, 20 °C, 3 h, 2. NaN_3, DMF, 60 °C, 18 h; b) Raney-Ni, H_2, 2-propanol, 20 °C, 12 h, 73 %; c) Fmoc-Val-OH, EEDQ, CH_2Cl_2, 20 °C, 12 h, 70 %; d) 1. TFA, 20 °C, 2 h, 2. Br-HYCRON-O*t*Bu (ref. [9]), NBu_4Br, $NaHCO_3$/CH_2Cl_2, 20 °C, 12 h, 75 %; e) TFA, 20 °C, 2 h, 95 %; f) H-AMPS, TBTU, HOBT, NMM, DMF, 20 °C, 18 h 74 %; g) morpholine/DMF (1/1); h) Fmoc-Pro-Ser(*t*Bu)-OH, TBTU, HOBT, NMM, DMF; i) 4 times [1. coupling: Fmoc-AS-OH, TBTU, HOBT, NMM, DMF; 2. capping: Ac_2O/pyridine (1/3); 3. Fmoc-removal: morpholine/DMF (1/1)]; j) Ac_2O/pyridine (1/3); k) [$Pd(PPh_3)_4$], morpholine, DMF/DMSO (1/1) 44 % based on **15**. Mes=βmorpholinylethanesulfonic acid, EEDQ = 2-ethoxy-*N*-ethoxycarbonyl-1,2-didyroquinoline.



Scheme 1. a) AcCl, 20 °C, 3 d, quant.; b) KCS_2OEt, EtOH, 20 °C, 4 h, 73 %; c) CH_3SBr/AgOTf, CH_3CN/CH_2Cl_2 (2/1), −62 °C, 3 h, 60 %; d) Ac_2O, pyridine, 0 °C, 4 h, 84 %; e) TFA/CH_2Cl_2 (1/1), anisole, 20 °C, 6 h, quant. Ac = acetyl, Tf = trifluoromethanesulfonyl, TFA = trifluoroacetic acid.



Scheme 2. a) Morpholine/DMF (1/1); b) Boc-Ala-OH, TBTU, HOBT, NMM, DMF; c) 1. CH_2Cl_2/TFA (1/1) 2. DIPEA, CH_2Cl_2; d) ten times [1. 2.–8., 10., and 11. coupling: Fmoc-AS-OH, TBTU, HOBT, NMM, DMF; 9. coupling: **7**; 2. capping: Ac_2O/pyridine (1/3); 3. Fmoc-removal: morpholine/DMF (1/1)]. Boc = *tert*-butoxycarbonyl, HOBT = hydroxybenzotriazole, TBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate, NMM = N-methylmorpholine, DIPEA = diisopropylethylamine, Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl, Fmoc = 9-fluorenylmethoxycarbonyl.

The TTX–T cell epitope–spacer conjugate **17** was obtained by Pd^0-catalyzed cleavage of the allylic anchor.[9] This fragment was coupled to the solid-phase-bound MUC1 glycododecapeptide after carboxy-group activation using HATU/HOAT[19] to furnish the T-cell epitope–spacer–MUC1 antigen conjugate **18** (Scheme 4).

After Pd^0-catalyzed cleavage of the HYCRON anchor and purification by means of preparative HPLC, the protected T cell epitope–spacer–MUC1 antigen conjugate **19** was isolated in pure form (as judged by NMR spectroscopy).[20] Hydrogenolysis of the sialic acid benzyl ester, acidolysis of the side-chain protecting groups, and mild methanolysis of the O-acetyl groups gave the pure conjugate **20** (as analyzed by HPLC and NMR spectroscopy)[21] in 47 % yield. Compound **20** corresponds to the delineated construct **1** containing the tetanus toxin epitope, a spacer, and the tumor-associated MUC1 glycopeptide antigen.

  1433-7851/01/4002-0367 $ 17.50+.50/0

H-G-V-T[α-Ac$_4$NeuNAc(OBn)(2-6)-α-Ac$_2$GalNAc]-S(*t*Bu)-A-P-D(*t*Bu)-T(*t*Bu)-R(Pmc)-P-A-P-O-HYCRON-β-Ala-AMPS

↓ a

9

Ac-Y(*t*Bu)-S(*t*Bu)-Y(*t*Bu)-F-P-S(*t*Bu)-V-Spacer-G-V-T[α-Ac$_4$NeuNAc(OBn)(2-6)-α-Ac$_2$GalNAc]-S(*t*Bu)-A-P-D(*t*Bu)-T(*t*Bu)-R(Pmc)-P-A-P-O-HYCRON-β-Ala-AMPS

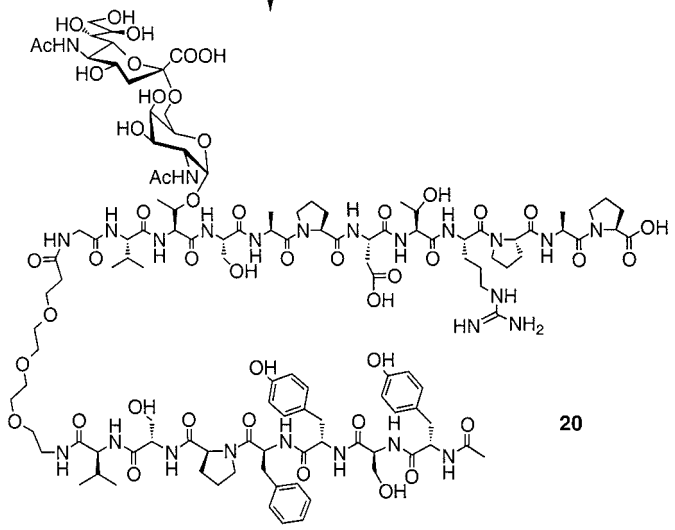
18

↓ b,c

Ac-Y(*t*Bu)-S(*t*Bu)-Y(*t*Bu)-F-P-S(*t*Bu)-V-Spacer-G-V-T[α-Ac$_4$NeuNAc(OBn)(2-6)-α-Ac$_2$GalNAc]-S(*t*Bu)-A-P-D(*t*Bu)-T(*t*Bu)-R(Pmc)-P-A-P-OH

19

↓ d



20

Scheme 4. a) **17**, HOAT/HATU/NMM, DMF, 20 °C, 2 d, 42 %; b) Ac_2O/pyridine (1/3); c) [Pd(PPh$_3$)$_4$], morpholine, DMF/DMSO (1/1) 20 % based on **8;** d) 1. Pd/C (10 %), H_2, MeOH, 20 °C, 1 h 2. CH_2Cl_2/TFA/thioanisole/ethanedithiol (10/10/1/1), 20 °C, 2 h 3. NaOMe, MeOH, pH 8.5, 20 °C, 18 h, 47 % over 3 steps. HOAT = 7-aza-1-hydroxy-1*H*-benzotriazole, HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazol[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate.

For immunological evaluation, conjugate **20** and, for comparison, the MUC1 dodecapeptide, the MUC1 ST_N glycododecapeptide of idenical sequence, the ST_N threonine conjugate, and the tetanus toxin–spacer conjugate (all in completely deblocked form) were applied to approximately 10^5 peripheric blood lymphocytes (PBL) from four healthy donors in concentrations of 0.1 μg and 1 μg per200 μL. Since a primary stimulation of the cells did not occur, restimulation with the same antigen was carried out after seven days until centers of proliferation appeared. According to an ELISA with murine antihuman IFNγ antibodies, cells from three of the four donors produced interferon-γ after the second restimulation with all these substances and before the T-cell growth factor interleukin 2 was added. After the fourth restimulation, the proliferation of the PBLs was measured by [^{3}H]thymidine incorporation. Proliferation was found to a different extent for the cells from each donor with all of the five substances, and also in a medium free of fetal calf serum. However, the proliferation only proceeded in the presence of antigen-presenting cells (APCs) and was not found for purified T-cells. This is considered as distinct proof of an antigen-specific reactivity. The proliferating lymphocytes were characterized using monoclonal antibodies directed against surface antigens in a fluorescence-activated cell sorter (FACS analysis). The analyses showed that conjugate **20** induces proliferation of up to 100 % for CD3-positive T-cells, and amongst those for 53 % of CD8-positive T-cells. The other substances also induced proliferation of CD3-positive T-cells, but a much lower extent of proliferation for the desired cytotoxic CD8-positive T-cells. These preliminary results justify the anticipation that conjugates of T cell epitopes and tumor-associated MUC glycopeptide antigens such as **20** can induce cytotoxic T-cell response[22] and that on this basis efficient synthetic antitumor vaccines can be developed.

Received: August 9, 2000 [Z 15608]

[1] a) T. Feizi, *Nature* **1985**, *314*, 53; b) G. F. Springer, *Science* **1984**, *224*, 1198.

[2] Reviews: a) S. J. Danishefsky, J. R. Allen, *Angew. Chem.* **2000**, *39*, 882; *Angew. Chem. Int. Ed.* **2000**, *112*, 836; b) R. R. Koganty, M.-A. Reddish, B. M. Longenecker in *Glycopeptides and Related Compounds* (Eds.: D. G. Large, C. D. Warren), Marcel Dekker, New York, **1997**, p. 707; c) H. Kunz, P. Wernig, M. Schilling, J. März, C. Unverzagt, S. Birnbach, U. Lang, H. Waldmann, *Environ. Health Perspect.* **1990**, *88*, 247.

[3] Review: S.-I. Hakomori, Y. Zhang, *Chem. Biol.* **1997**, *4*, 97.

[4] H. Kunz, S. Birnbach, *Angew. Chem.* **1986**, *98*, 354; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 360. T_N antigen = Ser/Thr (αGalNAc).

[5] W. Dippold, A. Steinborn, K.-H. Meyer zum Büschenfelde, *Environ. Health Perspect.* **1990**, *88*, 255.

[6] W. Dippold, A. Steinborn, S. Birnbach, H. Kunz, unpublished results; A. Steinborn, PhD Thesis, University Mainz, **1990**.

[7] Review: P. L. Nguyen, G. A. Niehans, D. L. Cherwitz, Y. S. Kim, S. B. Ho, *Tumor Biol.* **1996**, *17*, 176.

[8] S. J. Gendler, C. A. Lancaster, J. Taylor-Papadimitriu, T. Duhig, N. Peat, J. Burchell, L. Pemberton, E. N. Lalani, D. Wilson, *J. Biol. Chem.* **1990**, *265*, 15286.

[9] O. Seitz, H. Kunz, *Angew. Chem.* **1995**, *107*, 901; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 803.

[10] a) M. Leuck, H. Kunz, *J. Prakt. Chem.* **1997**, *339*, 332; b) P. Braun, G. M. Davies, M. R. Price, P. M. Williams, S. B. J. Tendler, H. Kunz, *Bioorg. Med. Chem.* **1998**, *6*, 1531.

[11] B. Liebe, H. Kunz, *Angew. Chem.* **1997**, *109*, 629; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 618.

[12] Other groups have also synthesized mucin glycopeptides with T_N–, T– and ST_N–antigen saccharide portions, see, for example, a) E. Meinjohanns, M. Meldal, A. Schleyer, H. Paulsen, K. Bock, *J. Chem. Soc. Perkin Trans. 1* **1996**, 985; b) S. D. Kuduk, J. B. Schwarz, X.-T. Chen, P. W. Glunz, D. Sames, G. Ragupanti, P. O. Livingston, S. J. Danishefsky, *J. Am. Chem Soc.* **1998**, *120*, 12474; c) J. B. Schwarz, S. D. Kuduk, X.-T. Chen, D. Sames, P. W. Glunz, S. J. Danishefsky, *J. Am. Chem Soc.* **1999**, *121*, 2662.

[13] a) J. Burchell, J. Taylor-Papadimitriu, M. Boshell, S. Gendler, T. Duhig, *Int. J. Cancer* **1989**, *44*, 691; b) M. R. Price, F. Hudecz, C. O'Sullivan, R. W. Baldwin, P. M. Edwards, S. B. J. Tendler, *Mol. Immunol.* **1990**, *62*, 795.

[14] K. Furuhata, K. Kimio, H. Ogura, T. Hata, *Chem. Pharm. Bull.* **1991**, *39*, 255.

[15] A. Marra, P. Sinaÿ, *Carbohydr. Res.* **1989**, *187*, 36.

[16] Trihydrate of **7**: $[\alpha]_D^{22} = 29.1$ ($c = 1.0$, MeOH). MALDI-MS (MeOH, dihydroxybenzoic acid (DHB)): *m/z*: 1239.1 [$M - H^+ + K^+ + Na^+$], 1223.3 [$M - H^+ + 2Na^+$], 1217.0 [$M + K^+$], 1201.5 [$M + Na^+$]; ^{1}HNMR (^{1}H-^{1}H COSY) (400 MHz, CD_3OD, TMS): $\delta = 4.99$ (dd, 1H, $J_{2,3} =$

  1433-7851/01/4002-0368 $ 17.50+.50/0

11.7 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 4.90 – 4.84 (m, 2H, H-1, H-4′), 4.69 (dd, 1H, J = 6.2, 10.9 Hz, OCH_2Fmoc), 4.51 (dd, 1H, J = 5.9, 10.9 Hz, OCH_2Fmoc), 4.10 (dd, 1H, J = 5.3, 12.3 Hz, H-9′b), 3.84 (dd, 1H, $J_{5,6a}$ = 7.5 Hz, $J_{6a,6b'}$ = 10.3 Hz, H-6a), 3.15 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 10.3 Hz, H-6b), 2.69 (dd, 1H, $J_{3'e,3'a}$ = 12.6, $J_{3'e,4'}$ = 4.4 Hz, H-3′e), 1.22 (d, 3H, J = 6.5 Hz, T^{γ}); ^{13}CNMR (DEPT) (100.6 MHz, CD_3OD): δ = 100.77 (C-1), 99.93 (C-2′), 38.83 (C-3′), 19.32 (T^{γ}).

[17] M. Gewehr, H. Kunz, *Synthesis* **1997**, 1499.

[18] J. Habermann, H. Kunz, *Tetrahedron Lett.* **1998**, *39*, 265.

[19] L. A. Carpino, A. S. El-Faham, C. Minor, F. Albericio, *J. Chem. Soc. Chem. Commun.* **1994**, 210.

[20] **19**: $[\alpha]_D^{22} = -20.8$ (c = 1.0, CH_3CN). FAB-MS (nitrobenzyl alcohol (nba), positive ion): m/z (%): 3754.4 (62.3) [$M(2\times{}^{13}C)-4\times$(*tert*-butyl)+2 × Na^+], calcd: 3753.74, 3755.1 (100) [$M(3\times{}^{13}C)-3\times$(*tert*-butyl)+2 × Na^+], 3755.8 (82.7) [$M(4\times{}^{13}C)-4\times$(*tert*-butyl)+2 × Na^+], 3757.2 (49.1) [$M(5\times{}^{13}C)-4\times$(*tert*-butyl) + 2 × Na^+]; ^{1}HNMR (400 MHz, [D$_6$]DMSO, TMS): δ = 8.48 – 7.41 (m, 18H, NH), 7.38 (m_c 5H, H_{Ar}-Bn), 7.28 – 7.16 (m, 5H, H_{Ar}-F), 7.11 (d, 2H, J = 7.9 Hz), 7.03 (d, 2H, J = 7.6 Hz, 2 × (H2-Y, H6-Y)), 6.82 – 6.76 (m, 4H, 2 × (H3-Y, H5-Y)), 6.70, 6.55 (S_{br}, $R^{NH(\varepsilon,\xi,\eta)}$), 5.22 – 5.14 (m, 5H, H-4, H-7′, H-8′, CH_2Bn), 4.92 – 4.85 (m, 2H, H-1, H-3); 4.78 – 469 (m, H-4′), 4.65 – 3.24 (m, 58H, F^{α}, 2 × Y^{α}, 2 × V^{α}, 3 × S^{α}, 3 × S^{β}, $CH_2CH_2(OCH_2CH_2CH_2)_2$= CH_2CH_2, R^{α}, D^{α}, 2 × A^{α}, 4 × P^{α}, 2 × T^{α}, G^{α}, V^{α}, H-2, H-5, H-6′, H-9′, 2 × T^{β}, 4 × P^{δ}, H-6a, H-5′), 3.22 – 2.60 (m, 10H, F^{β}, 2 × Y^{β}, H-6b, R^{δ}, H-3′e), 2.57 – 2.38 (m, 4H, D^{β}, CH_2CH_2Pmc), 2.38 – 2.35 (m, 2H, CH_2CONH-G-V), 2.49, 2.46 (s, 6H, *o,o′*-CH_3Pmc), 2.18 – 1.58 (m, 18H, 4 × P^{β}, 4 × P^{γ}, CH_2CH_2Pmc), 2.03, 2.00, 1.99, 1.93, 1.91, 1.85, 1.82, 1.75, 1.71, 1.66 (10 × s, 30H, m-CH_3Pmc, 6 × OAc, 3 × NHAc), 1.53 – 1.02 (m, 9H, 2 × V^{β}, R^{γ}, R^{β}, H3′a, 2 × A^{β}), 1.35, 1.23, 1.20, 1.15, 1.11, 1.08, 1.06, 1.05, (s, 69H, 7 × C(CH_3)$_3$, C(CH_3)$_2$Pmc), 0.98 – 0.90 (m, 3H, T_1^{γ}), 0.89 – 0.83 (m, 3H, T_2^{γ}), 0.82 – 0.78 (m, 12H, V^{γ}); ^{13}CNMR (DEPT) (100.6 MHz, [D$_6$]DMSO): δ = 98.40 (C-1), 98.01 (C-2′), 41.18, 40.97, 38.40, 36.95, 35.87 (2 × Y^{β}, F^{β}, C-3′, D^{β}, R^{δ}, CH_2CONH), 32.12 (CH_2CH_2Pmc), 19.05, 18.98, 18.75, 18.75, 18.16, 18.07, 17.95 (2 × V^{γ}, T_1^{γ}, *o,o′*-CH_3Pmc), 16.77 16.72, 16.00 (T_2^{γ}, 2 × A^{β}), 11.82 (m-CH_3Pmc).

[21] **20**: $[\alpha]_D^{22} = -76.9$ (c = 1.0, H_2O); FAB-MS (nba + LiBr, positive ion): m/z (%): 2770.7 (0.62) [M+Li^+], calcd: 2770.30; ^{1}HNMR (^{1}H-^{1}H COSY) (400 MHz, D_2O): δ = 7.37 – 7.24 (m, 3H), 7.23, 7.16 (m, 2H, H_{Ar}-F), 7.08 – 6.97 (m, 4H, 2 × (H2-Y, H6-Y)), 6.81 – 6.72 (m, 4H, 2 × (H3-Y, H5-Y)), 4.90 (m_c, 1H, H-1), 4.72 – 4.66 (m, 1H, D^{α}), 4.64 – 4.59 (m, 2H, F^{α}, R^{α}), 4.57 – 4.50 (m, 1H, A_1^{α}), 4.49 – 4.42 (m, 6H, A_2^{α}, 2 × S^{α}, 2 × Y^{α}), 4.41 – 4.33 (m, 6H, 4 × P^{α}, S_3^{α}, T_1^{α}), 4.32 – 4.29 (m, 2H, V_1^{α}, T_2^{α}), 4.28 – 4.23 (m, 1H, T_1^{β}), 4.22 – 4.17 (m, 1H, T_2^{β}), 4.12 – 4.03 (m, 3H, H-2, V_2^{α}, S_1^{β}), 4.01 – 3.27 (m, 40H, H-3, H-4, H-5, H-6, H-4′, H-5′, H-6′, H-7′, H-8′, H-9′, NH(CH_2CH_2O)$_3$CH_2CH_2CONH, 2 × S^{β}, G^{α}, 4 × P^{δ}), 3.17 (m_c, 2H, R^{δ}), 3.10 – 2.99 (m, 1H, $F^{\beta a}$), 2.90 – 2.77 (m, 7H, 2 × Y^{β}, D^{β}, $F^{\beta b}$), 2.67 (dd, 1H, H-3′e), 2.60 – 2.54 (m, 2H, CH_2CONH-G-V-), 2.33 – 2.18 (m, 4H 4 × $P^{\beta a}$), 2.11 – 1.77 (m, 15H, 2 × V^{β}, 4 × $P^{\beta b}$, 4 × P^{ψ}, $R^{\beta a}$), 2.03, 2.01, 1.93 (s, 9H, 3 × NHAc), 1.76 – 1.59 (m, 4H, H-3′a, $R^{\beta b}$, R^{γ}), 1.34 (m, 6H, J = 6.5 Hz, 2 × A^{β}), 1.30 – 1.21 (m, 3H, T_1^{γ}), 1.15 (d, 3H, J = 6.2 Hz, T_2^{γ}), 0.97 – 0.85 (m, 12H, 2 × V^{γ}); ^{13}CNMR (DEPT) (100.6 Mhz, D_2O): δ = 99.62 (C-1), 99.20 (C-2′), 22.14, 21.94, 21.49, (3 × NHAc), 18.73, 18.39, 18.22, 17.54 (2 × T^{γ}, 2 × V^{γ}), 15.11, 14.92 (2 × A^{β}).

[22] For the importance of this key property, see Section 2 in ref. [2a], p. 885.

Synthesis and Membrane-Binding Properties of a Characteristic Lipopeptide from the Membrane-Anchoring Domain of Influenza Virus A Hemagglutinin**

Frank Eisele, Jürgen Kuhlmann, and Herbert Waldmann*

Key events in the establishment and progression of viral infection are attachment and fusion of the virus with the cell and budding of new virus particles from the infected cell. This complex multistep process is decisively influenced and determined by posttranslationally modified proteins embedded in the viral lipid bilayer. For instance, hemagglutinin from influenza virus A is glycosylated in the extracellular domain[1, 2] and S-palmitoylated next to the transmembrane region (Figure 1).[3, 4] The glycoprotein part is responsible for initiation of viral infection through selective binding to sialic acid receptors on the surface of the host cell. The lipid residues are required for the interaction between the cell membrane and the free capsid during budding of viral offspring[5, 6] and are thought to mediate protein – protein and protein – lipid interactions in the viruses.[7] In addition, the lipidated regions may play an important role in fusion processes of the viral membrane with the endosome after entry of the viral particle into the cell.[8, 9] However, this proposal is controversial, since other investigations indicated that the lipidated cytoplasmic tail of the complex viral lipoglycoproteins is not essential for its membrane fusion activity.[10]

For the study of these and related processes in precise molecular detail, lipidated peptides which represent the characteristic linkage region between the protein backbone and the lipid groups and which additionally carry a marker by which they can be traced in biological systems may be employed as efficient molecular probes.[11] However, their synthesis is complicated by the pronounced base lability of the palmitic acid thioesters which hydrolyse spontaneously at pH > 7.[12] For the synthesis of such labile peptide conjugates enzymatic methods may open up viable alternatives to classical chemical techniques.[11] In this paper we report on the development of the *p*-phenylacetoxybenzyl (PAOB) ester, a new enzyme-labile protecting group for carboxyl function-

[*] Prof. Dr. H. Waldmann
Max-Planck-Institut für molekulare Physiologie
Abteilung Chemische Biologie
and Universität Dortmund
Institut für Organische Chemie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Fax: (+49) 231-133-2499
E-mail: herbert.waldmann@mpi-dortmund.mpg.de
Dr. J. Kuhlmann
Max-Planck-Institut für molekulare Physiologie
Abteilung Strukturelle Biologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Dr. F. Eisele
Universität Karlsruhe
Institut für Organische Chemie
Richard-Willstätter-Allee 2, 76128 Karlsruhe (Germany)

[**] This research was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.