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Accessory Publication

Membrane-based sensing approaches

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Chemicals:

α-*D*-hydroxyisovaleric acid (1) was prepared according to Losse and Bachmann[1] and benzyl- (2) or allyl-protected (6) with benzyl bromide or allyl bromide in DMF (Figure 2). The decadepsipeptide (13) was synthesized according to Gilon *et al.*[2] and 2,3-di-*O*-phytanyl-*sn*-glycerol-1-tetraethylene glycol-*D,L*-α-lipoic acid ester **(**DPhyTL) according to Schiller *et al.*. Protected Amino acids (Iris Biotech, Marktredwitz, Germany and NovaBiochem, Merck, Darmstadt, Germany), benzyl-*L*-lactic acid (3) (ABCR, Karlsruhe, Germany), 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine (DPhyPC, Avanti Polar Lipids, Alabaster, AL), palladium on activated charcoal (Pd/C, Fluka), *N,N'*-dicyclohexylcarbodiimide, Trifluoroacetic acid, 4-Dimethylaminopyridine, benzyl bromide, diphenylphosphoryl azide, potassium bicarbonate, potassium carbonate, dichloromethane, iron(III) chloride, hydroquinone, potassium chloride, sodium chloride, dimethylformamide, tetrachloromethane, oxalyl chloride, ferrocene carboxylic acid (16), *N,N*-diisopropylethylamine, phenylsilane, tetrakis(triphenylphosphine) palladium, ethanol (HPLC grade) and allyl bromide were used as received.

Analytical data for Fc-Valinomycin:

HPLC (60-100%, H_2O:MeOH + 0.1% TFA, 10 min): t_R = 15.5 min; ^{1}H-NMR (250 MHz, CD_2Cl_2): □ = 8.00 – 7.27 (m, 6H, 6 x NHCO), 5.24 – 4.95 (m, 6H, 6 x -CH-O-), 4.59 (m, 2H, ferrocenyl-CH), 4.24 (m, 2H, ferrocenyl-CH), 4.10 (s, 5H, ferrocenyl-CH), 3.76 – 4.09 (m, 6H, 6 x -CH-N-), 3.23 (m, 2H, $-CH_2-NH-^{Lys}$), 2.20 (m, 8H, $-CH^{Val, Hyiv}$), 1.94 (m , 4H, 2 x $-CH_2-^{Lys}$), 1.52 (m, 2H, $-CH_2-^{Lys}$), 1.37 (m, 9H, 3 x $-CH_3^{Lac}$), 0.89 (m, 48H, 16 x $-CH_3^{Val, Hyiv}$); ESI-MS: *m/z* found: 1374.6 $[m+Na^+]^+$; 1390.6 $[m+K^+]^+$; calcd: 1390.61 $[m+K^+]^+$.

Analytical instrumentation:

^{1}H-spectra were recorded on a Bruker AC 250 spectrometer and analyzed with Mestre-C. Mass spectroscopy was performed on a LCT-spectrometer Q-TOF Ultima 3. HPLC was measured with an Agilent 1100.

Electrochemical impedance spectroscopic (EIS) measurements were conducted on a Zahner spectrometer IM6 (Zahner Messtechnik, Kronach, Germany). Spectra were recorded for frequencies between 3 mHz and 100 kHz at 0 V bias potential with an ac modulation amplitude of 10 mV. Raw data were analyzed with the ZVIEW software package (Version 3.1, Scribner Associates). Three electrode measurements were performed with the substrate as working electrode, a coiled platinum wire as counter electrode and a DRIREF-2 reference electrode (World Precision Instruments, Berlin, Germany, which has a potential of 0.2881 V with reference to the NHE). The customized Teflon cells had a buffer volume of 1 mL and an electrochemically active area on the substrates of 0.28 cm^2.

Assembly of the tBLM: Template-stripped gold (TSG, 50 nm) was prepared as described elsewhere.[3] DPhyTL monolayers were obtained by selfassembly (c = 0.2 mg mL^{-1} in EtOH, 24 h), small unilamellar DPhyPC vesicles (50 nm by extrusion) were fused with the performed monolayers by addition of 25 □l of vesicle suspension (c = 2 mg mL^{-1}) in ultrapure water.

Fc-Valinomycin (c = 2 mg mL-1 in EtOH) was added to the bilayer to give a final concentration of 36 µM in the cell. Incorporation of the ion carrier was allowed to proceed for 2 h, followed by rinsing with 0.1 M KCl solution.

Oxidation and reduction of the Fc-Valinomycin was performed in the measuring cell by removing the electrodes, rinsing with 0.1 M iron(III) chloride or hydroquinone (+ 0.1 M KCl) and waiting for 24 h. Then the redox solution was rinsed out with the buffer solution.

References

[1] G. Losse, G. Bachmann, *Chem. Ber.* **1964**, *97*, 2671. doi:10.1002/cber.19640970935

[2] C. Gilon, Y. Klausner, A. Hassner, *Tetr. Letters* **1979**, *20*, 3811. doi:10.1016/S0040-4039(01)95531-5

[3] V. Atanasov, P. P. Atanasova, I. K. Vockenroth, N. Knorr, I. Köper, *Bioconjug. Chem.* **2006**, *17*, 631. doi:10.1021/bc050328n