New cationic amphiphilic compounds as potential antibacterial agents

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General Materials & Methods

Chemicals

Chemicals used in the reactions described in this thesis were obtained from the following commercial sources and used as received, unless otherwise stated (see last section of this paragraph). Chemicals were stored at temperatures indicated by the supplier and under inert atmospheres and dry conditions where necessary.

Acros: 30% AcOOH/AcOH, 1-adamantaneacetic acid, 29% ammonium hydroxide, Boc_2O, 1-bromodecane, 1-bromododecane, 1-bromoheptane, 1-bromohexadecane, 1-bromooctadecane, 1-bromooctane, 1-bromoundecane, butyric acid, chloranil, DAST, DIAD, 1,10-dibromodecane, 1,9-dibromononane, DIC, 2,2'-dithiobispyridine, DMAP, ethylene glycol, glycerol, heptanoic acid, hexanoic acid, HFIP, hydrazine hydrate, ICH_2CN, $LiAlH_4$, 2-mercaptoethanol, 1-methylimidazole, MMTCl, $NaBH_4$, nonanoic acid, octanoic acid, sodium thiophenolate, TFE, TMSCl, valeric acid. **Aldrich**: Ag_2SO_4, Ag_3PO_4, 1-bromononane, 1-bromotridecane, EDC.HCl, ethyl-3-mercaptopropionate, 20% phosgene/toluene. **Applied Biosystems**: HATU. **Bachem**: Fmoc-*N*-Me-Arg(Mtr)-OH, Fmoc-*N*-Me-Ser(tBu)-OH, 3-maleimidopropionic acid. **Baker**: $Na_2PO_4.12H_2O$ (z.A.). **BDH**: cetyltrimethylammonium bromide. **Biosolve**: MeCN (HPLC-S gradient grade), DiPEA, DMF, NMP, piperidine, TFA (peptide synthesis grade), Ac_2O, $CHCl_3$, CH_2Cl_2, 1,4-dioxane, DMSO, Et_2O, pyridine, tBuOH, THF (AR), MeOH (absolute HPLC), DCE (DNA synthesis grade). **Boom**: NaOH. **Bruker Daltonik:** α-cyanohydroxycinnamic acid (recrystalized). **Bufa**: $NaHCO_3$, $MgSO_4$. **Cambridge Isotopes**: acetone-d_6, $CDCl_3$, aq. DCl in D_2O, DMSO-d_6, D_2O, MeOD, TFE-d_3. **Fluka**: Boc-Cys(StBu)-OH, (*R*)-citronellyl bromide, DTT, Fmoc-βHSer(tBu)-OH, Fmoc-βHTyr(tBu)-OH), Fmoc-Cys(StBu)-OH, Fmoc-Mamb-OH, Fmoc-Pamb-OH, MS 3Å, MS 4Å, 1M PMe_3/toluene, PyBroP, TCEP·HCl, TentaGel PHB (0.24mmol/g), TentaGel S NH_2 (0.26mmol/g), TIS, 1% TNBS/DMF. **FTI**: fluorous silica gel, [1H,1H,2H,2H]-perfluorodecyl iodide, [1H,1H,2H,2H]-perfluoroundecanoic acid. **ICN**: basic alumina. **Iris Biotech**: HCTU. **Merck**: AcOH (glacial), NH_4Oac, silica gel. **Molecular Probes**: DPX. **NeoSystem**: Boc-Dab(Fmoc)-OH, Fmoc -Abu-OH, Fmoc-Ava-OH, Fmoc-Capro-OH, Fmoc-Cmpi-OH, Fmoc-Dab(Boc)-OH, Fmoc-DPhe-OH, Fmoc-Sar-OH, Fmoc-Tran-OH, HBTU. **Nova Biochem**: Boc-Cys(Tr)-OH, 3-carboxypropanesulfonamide, Fmoc-βAla-OH, Fmoc-Dab(ivDde)-OH, Fmoc-Dab(Mtt)-OH, Fmoc-DSer(tBu)-OH, Fmoc-DTyr(tBu)-OH, Rink amide resin (0.64mmol/g), Wang resin (0.96mmol/g). **Perseptive Biosystems**: HOAt. **Riedel-de Haën**: EtOAc, PE 40/60 (puriss.), toluene (purum). **Senn Chemicals**: AM resin (0.36mmol/g), BOP, Fmoc-Abu-OH, Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Asn(Tr)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Tr)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ava-OH, Fmoc-Cys(Tr)-OH, Fmoc-Gly-OH, Fmoc-His(Tr)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Sar-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH, FmocOSu, HOBt, PyBOP

H_2O was purified using a Millpore MilliQ purification instrument. MeOH was stored on MS 3Å, CH_2Cl_2 and DMF on MS 4Å. THF was distilled prior to use from $LiAlH_4$ (5g/L). Toluene, PE and EtOAc used in sgcc and work-up procedures were distilled before use.

Analytical Techniques

TLC – Reactions were followed by TLC on silica gel (Schleider & Schull F 1500 LS 254) or HPTLC sheets (Merck, silica gel 60, F 254), with detection by UV absorption (254nm) where applicable followed by charring at 150^0C after spraying with ninhydrin (3g/L) in EtOH/AcOH (100/3 v/v), 20% H_2SO_4 in EtOH (25g/L), $NH_4Mo_7O_{24}{\cdot}4H_2O$ (25g/L) and $NH_4Ce(SO_4)_4{\cdot}2H_2O$ (10g/L) in 10% aq. H_2SO_4, or 2% $KMnO_4$ in 1% aq. K_2CO_3.

NMR – ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and ^{31}P NMR were recorded on Bruker AC200 (200MHz), Bruker DPX-400 (400MHz) and/or Bruker DMX-600 (600MHz). H-H COSY, C-H COSY, TOCSY, ROESY and NOESY spectra were recorded on a Bruker DMX-600 (600MHz). Chemical shifts are in ppm relative to tetramethylsilane as internal standard at 0.0ppm (^{1}H NMR) or relative to shifts of deuterated solvents (^{13}C NMR) according to literature values ($CDCl_3$ middle resonance at 77.0ppm)

HPLC – RP-HPLC analyses and purifications were performed on a ÄKTA Explorer HPLC system. Alltech Alltima C_{18} analytical (250x4.6mm) and semi-preparative (250x10.0mm) columns were used. Applied buffer systems: maximal gradient of 5→80% MeCN in 0.1% aq. TFA. Eluents were degassed before use with helium.

(LC)MS – Analyses were performed using a Jasco 900 LC system with simultaneous detection at 214 and 254nm connected to a Perkin Elmer SCIEX API 165 Q-TOF monoquad ESI mass spectrometer in positive ion mode. The applied LC buffer system runs with a maximal gradient of 10→90% MeCN in 0.1% aq. TFA (eluents were degassed with helium) over an Alltima Alltech analytical C_{18} column (150x4.6mm) at 1mL/min. LCMS samples were dissolved in mixtures containing any of AcOH, H_2O, MeCN, tBuOH, TFA, TFE, MeOH and HFIP, and for MS any of MeCN, MeOH, DMSO, DMF or H_2O, where applicable.

HRMS – HRMS spectra were recorded on an API QSTAR Pulsar (Applied Biosystems) or TSQ Quantum (Thermo Finnigan) fitted with an acurate mass option, interpolating between PEG peaks.

MALDI-MS – MALDI-MS analyses were performed on a Bruker Biflex III reflectron TOF mass spectrometer, equipped with delayed extraction and with a UV ionization laser (N_2, 337nm). For the analyses in Chapter 1, 5µL amounts of peptide samples were mixed with 5µL matrix solution (sat. α-cyanohydroxycinnamic acid with 0.1% TFA in MeCN/H_2O 1/1 (v/v)). Of this matrix mixture, 0.5µL was brought on the target plate.

Determination of loading of resin with first amino acid – The loading of resin with the first amino acid was determined by photospectrometric detection. To this end, a sample of resin 2-5mg was treated for 10min 20% piperidine/DMF (1mL), then the total volume was adjusted to 10mL by addition of MeOH, and UV-absorption was measured at 300nm in a 1cm cuvet (Perkin Elmer DMS 200 spectrophotometer). The loading was caluclated using the expression below derived from Lambert-Beer's Law where L is the loading in mmol/g, A_{300} the absorbance at 300nm, vol the initial volume of the sample (10mL) and wt is the weight of the resin sample in mg.

$L=(A_{300} \times vol)/(7.8 \times wt)$

Kaiser test[1] – For identification of free primary amines, the Kaiser test was performed: to 2-3mg of resin, 20µL of the three solutions were added (5g ninhydrin in 100mL EtOH; 80g PhOH in 20mL EtOH; 2mL 0.001M KCN in 98mL pyridine) and the mixture heated to 120°C for 5min. A blue/purple resin colour indicates a positive test.

TNBS analysis[2] – As alternative to the Kaiser test, the TNBS test for identification of primary amines was applied. To this end, 20µL of 1% DiPEA/DMF and 20µL of 1% TNBS/DMF were added to samples of 2-3mg of resin. A red/orange resin colour after standing for 5min at RT indicates a positive test.

Chloranil analysis[3] – For identification of secondary amines, the chloranil test was applied: to a 2-3mg sample of resin, 20µL of 1% chloranil/DMF and 20µL of 1% DiPEA/DMF were added and the sample allowed to react for 5min at RT. Coloured resin indicates a positive test.

Solid-Phase Peptide Synthesis

Peptides were prepared by using automated Fmoc-based SPPS custom protocols using any of the following synthesizers; ABI433A (Applied Biosystems), Syro 2000 (MultiSyntech) or 336 System 3 Peptide Synthesizer (CS Bio). Standard cycles (of which the duration differed for the three synthesizers, but followed customized programs) for acylating preloaded Wang or Rink amide resins commenced with washing of the resin with NMP (and swelling with CH_2Cl_2 in the first cycle), followed by a Fmoc deprotection step applying 20% piperidine/NMP. In general. acylation was effected by using 3-6eq. of Fmoc-amino acid/DiPEA (6-12eq.), with PyBOP (Syro 2000, in NMP with LiCl) or HCTU (ABI433A and CS Bio) in NMP or NMP/DMF 1/1 (v/v). Less often used activators include BOP, HBTU and HATU. After coupling, the resin was washed with NMP and a capping step comprising either Ac_2O/NMP, Ac_2O/DiPEA/NMP or Ac_2O/DiPEA/HOBt/NMP was applied. SPPS were performed with N_2 as pressurizing gas and resulted in on-resin peptides with either the last Fmoc removed (ABI433A, Syro 2000) or maintained (CS Bio). Synthesis scales ranged from 10-100µmol.

1. Kaiser, E.; Colescott, R.L.; Bossinger, C.D.; Cook, P.I.; *Anal. Biochem.* **1970**, *34*, 595
2. Hancock, W.S.; Battersby, J.E. *Anal. Biochem.* **1976**, *71*, 261
3. Vojkovsky, J. *Peptide Res.* **1995**, *8*, 236