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# Synthesis of Functionalized Guanidino Amino Acids

**Thomas Suhs and Burkhard König*[a]**

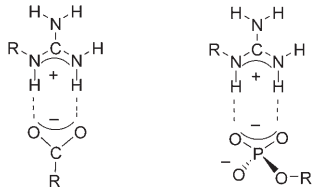
**Abstract:** We report the synthesis of guanidino amino acids (GuAA), which are structurally related to Arg and resemble a dipeptide consisting of α- and γ-amino acid with a guanidinium group in the main chain. The compounds are available with different protecting groups in gram amounts and are intended as synthetic building blocks for the construction of synthetic oxoanion or peptide receptors. Tyr, Trp or dansyl-functionalized Lys can be introduced as the α-amino acid part, which leads to luminescent GuAAs. The compounds signal carboxylate binding in MeOH, DMSO and buffered water by change of the emission intensity. The property may find use in the construction of chemosensors.

**Keywords:** amino acids · carboxylate binding · guanidinium · luminescence · receptors

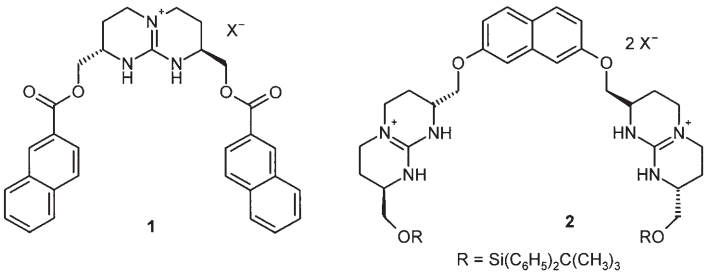
## Introduction

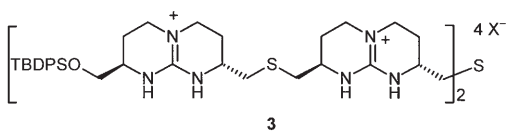
Arginine residues and their function as anion-binding sites are ubiquitous in nature. They are found in the binding region of a large number of enzymes and signalling proteins and interact with negatively charged anionic[1,2] or π-electron-rich aromatic moieties[3] of substrates or cofactors. The origin of the strong interaction with oxoanions is the binding pattern featuring two parallel hydrogen bonds in addition to the electrostatic interaction (Scheme 1).[4,5] Strong basicity of guanidine ($pK_a$ 13.5) ensures protonation over a wide pH range.[6] The guanidinium functionality was utilized by molecular recognition chemists as a strong and selective binder for anionic guests.[4,7–9] Strong binding interactions in competitiv'e media were reported for receptors containing this functionality. Due to the advantageous properties of the guanidinium group, it was used in receptor designs for almost every class of small anionic target molecules.

Lehn was among the first to investigate the use of guanidinium salts in the complexation of carboxylates.[10] Schmidtchen,[11–15] Lehn and de Mendoza[16,17] used bicyclic guanidinium ions to develop anion receptors such as **1** and **2** (Scheme 2). Compound **1** is capable of extracting *p*-nitrobenzoate anions from aqueous media into organic solvents. The chiral structure of (*S*,*S*)-**1** enabled enantioselective differentiation of chiral carboxylate anions such as the sodium salts of (*S*)-mandelate and (*S*)-naproxenate. Similarly, recep-



Scheme 1. Binding pattern of guanidinium groups with carboxylate and phosphate anions as found in many X-ray structures of the corresponding salts.




Scheme 2. Examples of guanidinium ion based receptors.

[a] Dr. T. Suhs, Prof. Dr. B. König
Institut für Organische Chemie
Universität Regensburg (Germany)
Fax: (+49) 941-943-1717
E-mail: Burkhard.Koenig@chemie.uni-regensburg.de

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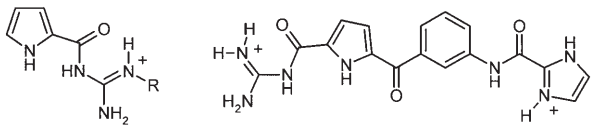
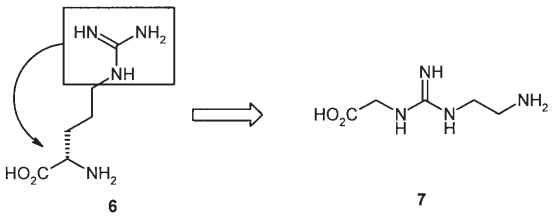
tors (*S*,*S*)-**1** and (*R*,*R*)-**1** were used to extract L- and *N*-acetyl-D-tryptophan from a racemic mixture of the L and D enantiomers. Compound **2** allows effective binding of dicarboxylates. Hamilton[18] and de Mendoza[19] extended the bicyclic guanidinium motif to synthetic receptor **3** that binds and stabilizes the α-helical conformation of glutamate- and aspartate-rich peptides.[20–23] More recently guanidinium receptors have been used as transfer vectors[24] in biology and for the self-assembly of fullerenes.[25] Earlier work investigated the use of guanidinium groups as catalysts for hydrolysis reactions[26–28] and Kilburn et al.[29–30] used diaminoguanidines as carboxylate ion binding sites in tweezer-type peptide receptors.

Incorporation of additional hydrogen-bonding functionality enhances the binding ability of simple guanidinium salts. Schmuck reported on guanidinocarbonyl pyrrols **4** (Scheme 3)[31,32] as efficient receptors for the complexation of amino acid carboxylates. A de novo designed guanidinocarbonyl receptor **5** for dipeptide binding in water was described by the same group.[33–34] The binding properties of **5** to a series of dipeptides were studied by UV titration in water and revealed lower micromolar affinities and selectivity.



Scheme 3. Guanidinocarbonyl pyrrol receptors **4** and **5** for amino acid and peptide binding.

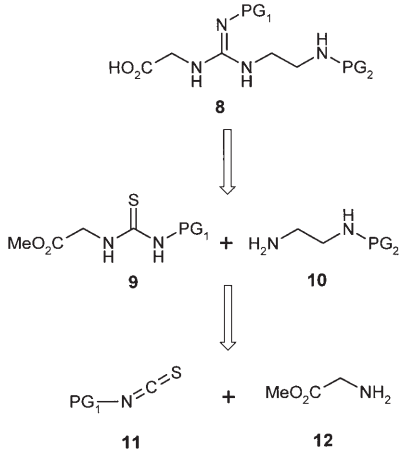
The few examples illustrate how useful the guanidinium group is as an oxoanion binding site in synthetic receptors. However, the synthesis of extended receptor structures around the guanidinium group may be tedious and typically requires individually optimized procedures. A guanidinium compound which is protected and suitably functionalized for peptide bond formation may facilitate guanidinium-based synthetic receptor synthesis. We have therefore developed synthetic routes to protected derivatives of the parent guanidino amino acid **7** (GuAA; Scheme 4), which may be considered as a structural analogue of arginine.



Scheme 4. Guanidino amino acid (GuAA) **7** as a versatile intermediate for the synthesis of guanidinium-based synthetic receptors.

## Results and Discussion

Compound **7** has been, to the best of our knowledge, described only once thus far in the literature within the search for antidiabetic aminoguanidines.[35] To facilitate incorporation of GuAAs into more extended receptor structures the guanidine group of **7** must be protected orthogonally to the primary amine function to avoid side reactions of the guanidine group in subsequent steps. Therefore, we developed a synthesis giving the compound in orthogonally protected form. Two routes for their synthesis have been established. Route A (Scheme 5) starts from isothiocyanate **11** reacting



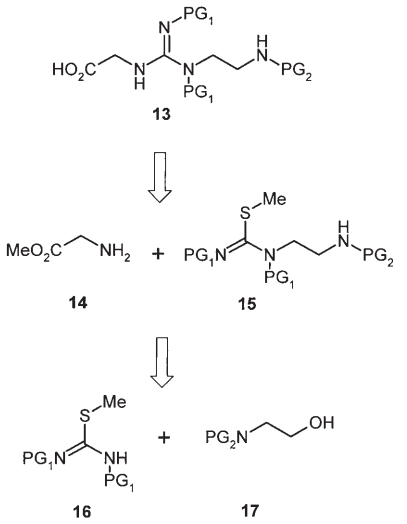
Scheme 5. Retrosynthetic analysis of route A to GuAA.

with glycine methyl ester to give thiourea **9**. Reaction with monoprotected 1,2-diamine **10** yields GuAA **8**. Route B (Scheme 6) gives access to a twofold protected guanidine moiety and starts from S-methyl thiourea **16**. Mitsunobu reaction with N-protected aminoalcohol **17** gives intermediate **15**, which is converted to the target structure **13** by reaction with amino acid esters.

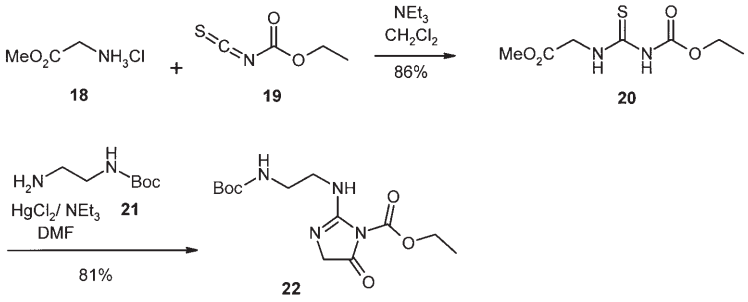
**Synthesis of protected GuAAs—route A**: Various methods exist for the synthesis of guanidine derivatives from different starting materials and reagents.[36] One of the well-known methods is the conversion of thioureas, protected with electron withdrawing groups such as Boc[37] or Cbz,[38] into guanidinium moities in the presence of a coupling reagent.[39] Ansyln and co-workers described the efficient synthesis of guanidinium derivatives using the ethyl carbamate protecting group and EDCI as the coupling reagent.[40] The advantages of the ethyl carbamate protecting group are its commercial availability of the starting material and rapid formation of the protected thiourea intermediate. We therefore use ethyl carbamate as protecting group $PG^1$ in our synthesis and started our approach with the formation of thiourea **20** obtained in very good yields. Transformation of **20** with mono-Boc-protected *N*,*N*′-ethylenediamine **21**[41] in the presence of $HgCl_2$ did not give the acyclic guanidinium

amino acid, but the cyclic guanidinium derivative **22** in good yields (Scheme 7).



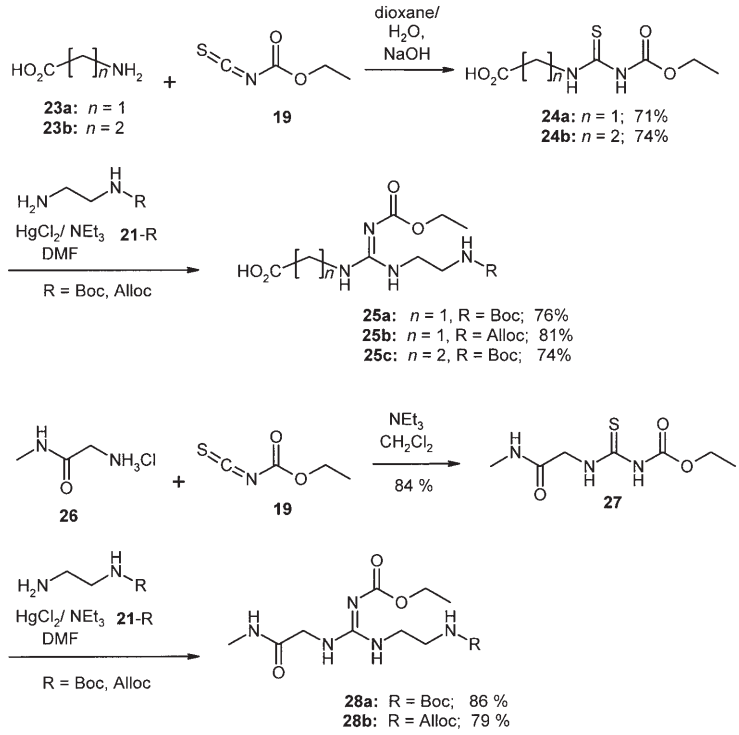
Scheme 6. Retrosynthetic analysis of route B to GuAA.



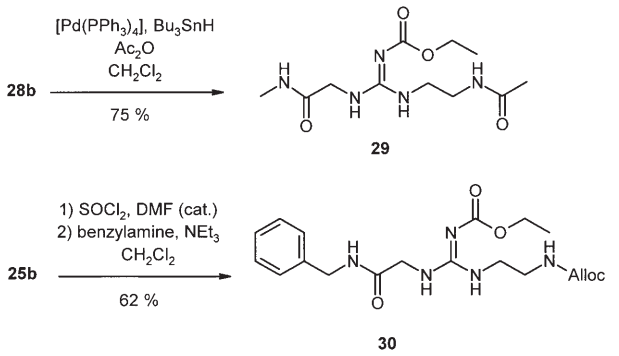
Scheme 7. Synthesis of the cyclic guanidine derivative **22**.

To avoid the cyclisation reaction the ester group was replaced by a carboxylic acid or amide functionality. Scheme 8 summarizes the synthesis of ethoxycarbonyl-protected GuAAs **25** and **28** with either Boc- or Alloc protecting groups on the primary amine. Palladium-catalyzed deprotection of the Alloc group using tributyltin hydride as nucleophile and the presence of carboxylic acid anhydrides, acid chlorides and activated esters allows acylation of the primary amine in one step (Scheme 9, top).[42] Transformation of the carboxylic acid is exemplarily shown by acid chloride formation and coupling with benzylamine to give compound **30** in 62% yield (Scheme 9, bottom).

Anslyn described the removal of the ethyl carbamate group using $Me_3SiBr$ under reflux in DMF followed by protonation with methanol to give the unprotected guanidinium compounds in high yields.[40] These conditions failed for our compounds and we therefore employed enzymatic hydrolysis with pig liver esterase (PLE, E.C.3.1.1.1).[43–44] The ethoxycarbonyl group of **28b** is readily cleaved upon treatment with PLE EC3.1.1.1 (Scheme 10). The Alloc group is stable under the reaction conditions.

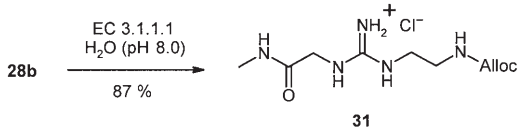


Scheme 8. Synthesis of ethoxy-carbonyl protected acyclic guanidinium amino acids **25** and guanidinium amino amides **28**.



Scheme 9. Transformations of amine- (top) and carboxy-terminus (bottom) of GuAAs.

The basicity of acylated guanidine groups, as in **28a**, is significantly reduced ($pK_a \sim 8$),[45] but they still provide two hydrogen bond donor sites for oxoanion binding. NMR titration of non-protonated guanidine **28a** with acetate ions in

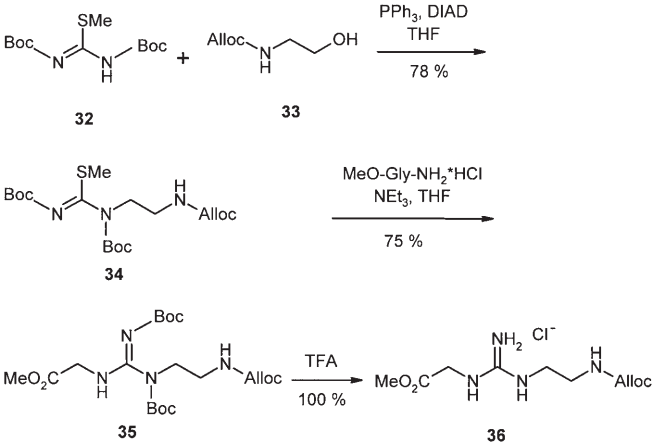


Scheme 10. Selective removal of the ethoxycarbonyl protecting group with pig liver esterase EC3.1.1.1.

DMSO revealed a binding constant $K_a = 1.98 \pm 0.4 \times 10^3\ L\,mol^{-1}$ from non-linear fitting of the chemical induced shift (CIS) of the NH protons (see Supporting Information for data). The value is comparable to the binding constants of thiourea to carboxylates.[46]

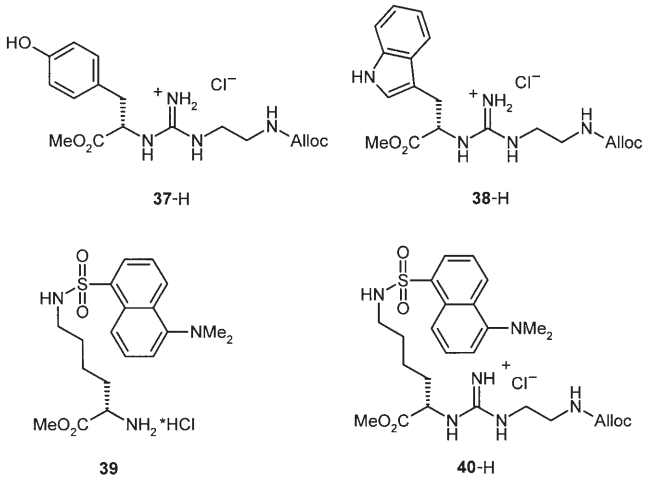
**Synthesis of protected GuAAs—route B**: The synthesis following route B starts with standard Mitsunobu reaction of N-Alloc-protected ethanolamine **33**[47] and 1,3-di-Boc-2-methyl-isothiourea **32**[48] to provide the alkylated isothiourea **34** in good yields (Scheme 11).[49] Reaction of the isothiourea **34** with glycine methyl ester in refluxing THF over night gave the expected guanidine **35** in 75% yield. The use of $HgCl_2$ for isothiourea activation is not necessary; yields go down to 45% if $HgCl_2$ is added. Cleavage of the two Boc groups with TFA/dichloromethane 1:4 and anion exchange against chloride give the target guanidinium amino ester **36**.




Scheme 11. Synthesis of guanidinium amino ester **36**.

The natural amino acids Tyr and Trp show emission around 303 nm (excitation 274 nm) and 350 nm (280 nm). If Gly is replaced in the conversion of isothiourea **34** by these amino acids or dansylated Lys **39**, fluorescent guanidinium compounds are obtained (Scheme 12). Such compounds are useful in the design of synthetic receptors, because they signal oxoanion binding by emission changes. The preparation of tyrosine guanidinium compound **37**-H, tryptophan guanidinium **38**-H and dansyl lysine guanidinium **40**-H follows the same route as shown in Scheme 11. The reaction yield of the amino acids with isothiourea **34** is with 56, 62 or 54%, respectively, somewhat lower compared with Gly.

The emission quantum yields of the fluorescent guanidinium amino esters **37**-H, **38**-H and **40**-H were measured in different solvents with quinine bisulfate as reference. The effect of solvent on the emission quantum yield is similar for all compounds decreasing with higher polarity. The quantum yields of the guanidinium compounds are lower compared with the quantum yield of the natural amino acids.[50] The data are summarized in Table 1.




Scheme 12. Synthesis of luminescent guanidinium compounds.

Table 1. Quantum yields of guanidinium amino esters **37**-H, **38**-H and **40**-H in %. Quinine bisulfate was used as standard; concentration: $5 \times 10^{-5}$.

| Compound | DMSO | MeOH | $H_2O$ |
|---|---|---|---|
| **37**-H | 13 | 13 | 10 |
| **38**-H | 18 | 14 | 13 |
| **40**-H | 15 | 14 | 7 |

To verify the binding affinities of the fluorescent guanidines **37**-H, **38**-H and **40**-H to carboxylates luminescence titrations in DMSO and $H_2O$ were performed.[51] Binding constants were derived by non-linear fitting of the decrease of emission intensity. The binding constants of **37**-H, **38**-H and **40**-H to acetate salts and N-protected amino acids are nearly identical (see Table 2) in the same solvent without selectivity between different amino acid side chains. The observed binding constants are slightly higher than reported data for simple guanidinium–carboxylate interactions (DMSO: $K_{11.} = 5 \times 10^3$ to $9 \times 10^3\ L\,M^{-1}$),[52–54] but previous studies have shown that the vicinity of aromatic π systems[55] or additional interactions[56] may enhance guanidinium–carboxylate ion interactions significantly. Therefore, a contribution of the phenol, indol and naphthyl substituents in **37**-H, **38**-H and **40**-H to the carboxylate binding may be envisaged. The isothermal calorimetric titration of **37**-H with sodium acetate in aqueous buffer confirms the binding affinity derived from emission titrations (see Supporting Information for data).

The selective deprotection of the GuAA building block for subsequent reactions is illustrated for compound **37**-Boc. Acylation of the primary amine function with acetic anhydride to **41**-Boc proceeds smoothly in 30 min and 84% using $[Pd(PPh_3)_4]$ (5 mol%) and $Bu_3SnH$ (1.1 equiv). Cleavage of the ester group with LiOH affords cleanly the corresponding guanidine-protected amino acids **42**-Boc and **43**-Boc (Scheme 13). A combination of the reactions allows the synthesis of a glycine-bridged bisguanidine compound **47**-Boc.

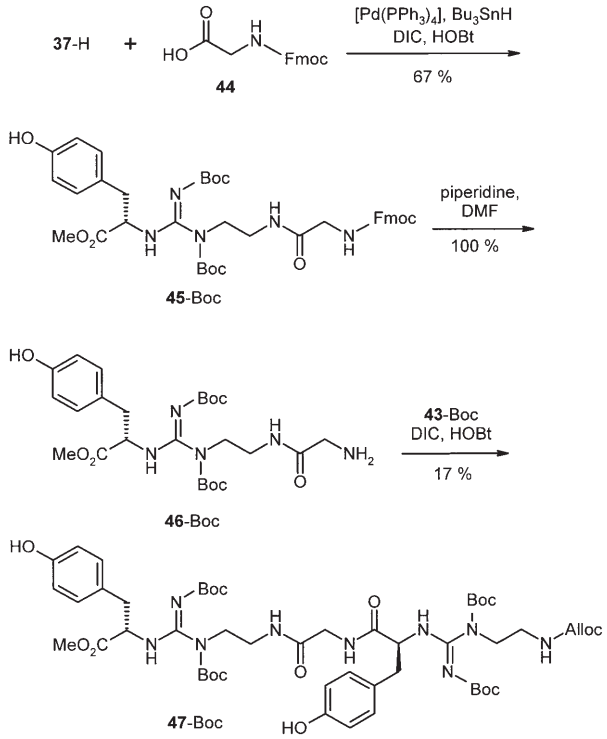
  

Table 2. Binding constants of luminescent guanidium compounds **37**-H, **38**-H and **40**-H to carboxylate salts in DMSO and buffered water (buffer concentration 50 mM). The stoichiometry of all aggregates is 1:1, as confirmed by Job's plot analysis (see Supporting Information).

| Guanidinium compound | Carboxylate salt | Solvent | $K_{11}$ [$L\,mol^{-1}$][a] |
|---|---|---|---|
| **37**-H | $Bu_4NOAc$ | DMSO | $2.5\times10^4$ |
| **37**-H | NaOAc | $H_2O$ (Tris, pH 7.0 | $7.9\times10^3$ |
| **37**-H | Boc-Gly-OH | $H_2O$ (Tris, pH 7.0 | $7.9\times10^3$ |
| **37**-H | Boc-Ala-OH | $H_2O$ (Tris, pH 7.0 | $7.9\times10^3$ |
| **37**-H | Boc-Val-OH | $H_2O$ (Tris, pH 7.0 | $7.9\times10^3$ |
| **38**-H | $Bu_4NOAc$ | DMSO | $2.4\times10^4$ |
| **38**-H | NaOAc | $H_2O$ (Tris, pH 7.0 | $7.9\times10^3$ |
| **40**-H | $Bu_4NOAc$ | DMSO | $2.2\times10^4$ |
| **40**-H | NaOAc | $H_2O$ (Tris, pH 7.0 | $7.8\times10^3$ |

[a] All binding constants have errors of approx. ±6%.

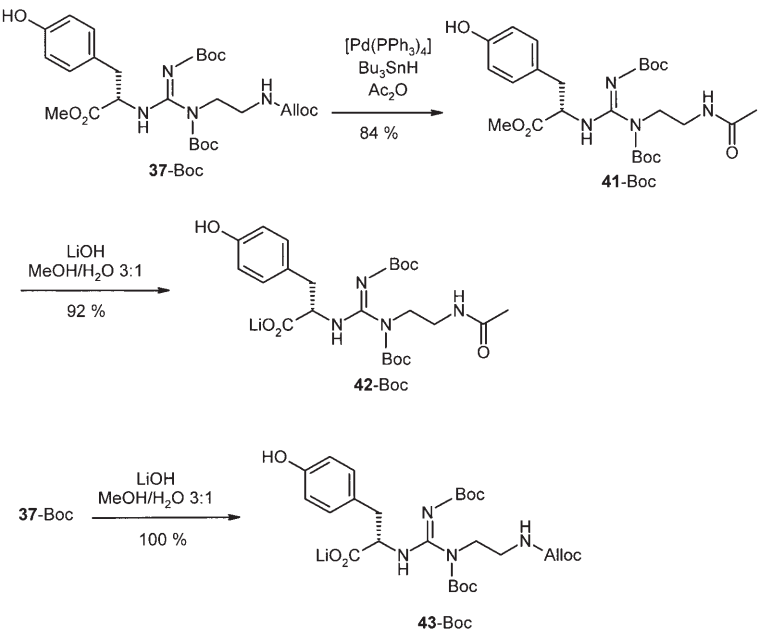
The guanidine **37**-Boc was acylated with Fmoc-glycine (**44**) in 67% yield and the Fmoc group removed using piperidine. Peptide coupling of the resulting **46**-Boc with **43**-Boc gave compound **47**-Boc, although only in low yield (Scheme 14).




Scheme 14. Synthesis of glycine-bridged bis-guanidine **47**-Boc.

## Conclusion

The guanidinium group is a widely used binding site motif in synthetic receptors for oxoanions and peptides. We report the preparation of guanidinium amino acids (GuAA) of the general structure **7** as versatile intermediates for the synthesis of guanidinium-based synthetic receptors. The compounds are available in gram amounts in protected form from simple starting materials. Selective removal of amine, carboxy and guanidine protecting groups was demonstrated, which allows a facile utilization in receptor synthesis. The GuAA **7** structure resembles a dipeptide of α- and γ-amino acid. Tyr, Trp or side chain dansyl-functionalized Lys can be employed as the α-amino acid part. This leads to luminescent GuAAs, which respond to carboxylate binding by emission changes and may find application in the preparation of chemo sensors.




Scheme 13. Deprotection of compound **37**-Boc.

## Experimental Section

**General methods**: All $^1H$ and $^{13}C$ NMR spectra were measured in [$D_6$]DMSO at 300 K with TMS as internal reference at 300 or 75.5 MHz, respectively, if not stated otherwise. Column Chromatography (CC) was performed on silica gel (70–230 mesh).

**3-Ethoxycarbonyl thiourea acetic acid (24a)**: Ethoxycarbonyl isothiocyanate (**19**) (1.16 g, 1 mL, 8.87 mmol) in dioxane (20 mL) was added to a solution of glycine (**23a**) (1 g, 13.3 mmol) in $H_2O$ (10 mL) and 1 N NaOH (5 mL). The mixture was stirred at room temperature for 12 h and the organic solvent was removed under reduced pressure. The water phase was acidified with 5% $KHSO_4$ solution to pH 2. After addition of EtOAc (25 mL) the phases were separated and the water phase was extracted twice with EtOAc (2×40 mL). The combined organic phases were dried over $Na_2SO_4$ and the solvent was removed under re-

duced pressure. The crude product was purified by CC (EtOAc; $R_f$ = 0.35) to give **24a** (1.29 g, 71%) as white solid. M.p. 78°C; $^1$H NMR: $\delta$ = 1.23 (t, $J$ = 7.1 Hz, 3H), 4.17 (q, $J$ = 7.1 Hz, 2H), 4.27 (d, $J$ = 5.2 Hz, 2H), 10.15 (brs, 1H), 11.14 (s, 1H), 13.0 ppm (brs, 1H); $^{13}$C NMR: $\delta$ = 14.1 (+), 46.4 (−), 61.7 (−), 153.3 ($C_{quat}$), 169.8 ($C_{quat}$), 179.9 ppm ($C_{quat}$); IR (KBr): $\tilde{\nu}$ = 3180, 2970, 2908, 1720, 1545, 1448, 1414, 1255, 1205, 1146 cm$^{-1}$; MS (EI, 70 eV): $m/z$ (%): calcd for $C_6H_{10}N_2O_4S$: 206.04; found: 206.1 (100) [$M^+$], 188.0 (16) [$M^+-H_2O$], 160.0 (23) [$M^+-C_2H_5OH$].

**[$N'$-(2-Allyloxycarbonylethyl)-$N''$-ethoxycarbonylguanidino] acetic acid (25b)**: Thiourea **24a** (513 mg, 2.48 mmol) was added to a solution of 1-allyloxycarbonylethyldiamine[42] (672 mg, 3.72 mmol) and $NEt_3$ (1.00 g, 1.37 mL, 9.92 mmol) in DMF (25 mL). Hg$^{II}$ chloride (809 mg, 2.98 mmol) was added in one portion and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in $H_2O$ (25 mL) and acidified with 5% $KHSO_4$ to pH 2–3. The aqueous layer was extracted with EtOAc (3×50 mL). The combined organic layers were dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude product was purified by CC (EtOAc; $R_f$ = 0.1) to give **25b** (635 mg, 81%), as a white solid. M.p. 104°C; $^1$H NMR: $\delta$ = 1.21 (t, $J$ = 7.1 Hz, 3H), 2.98–3.18 (m, 4H), 3.75 (d, $J$ = 5.2 Hz, 2H), 4.12 (q, $J$ = 7.1 Hz, 2H), 4.46 (d, 5.2 Hz, 2H), 5.17 (dd, $J$ = 1.7, 9.3 Hz, 1H), 5.27 (dd, $J$ = 1.7 Hz, 16.2 Hz, 1H), 5.83–5.97 (m, 1H), 7.23 (brs, 1H), 8.00–8.17 (m, 2H), 10.06 ppm (brs, 1H); $^{13}$C NMR: $\delta$ = 14.1 (+), 38.5 (−), 39.6 (−), 42.4 (−), 61.1 (−), 64.2 (−), 116.9 (−), 133.6 (+), 152.8 ($C_{quat}$), 154.3 ($C_{quat}$), 155.9 ($C_{quat}$), 168.6 ppm ($C_{quat}$); IR (KBr): $\tilde{\nu}$ = 3325, 3085, 2984, 2943, 1698, 1655, 1552, 1249, 1152, 1101, 1017, 912, 875, 763, 687 cm$^{-1}$; MS (CI, $NH_3$): $m/z$ (%): 334.1 (10) [$M^+$+$NH_4$], 317.1 (100) [$M^+$+H]; elemental analysis calcd (%) for $C_{12}H_{20}N_4O_6$ (316.14): C 45.57, H 6.37, N 17.71; found: C 45.24, H 6.35, N 17.76.

***N*-(Methylacetamid)-$N'$-(ethoxycarbonyl)thiourea (27)**: Ethoxycarbonyl isothiocyanate **19** (2.32 g, 17.7 mmol) was added to a solution of glycine-methylamide (**26**) (3.3 g, 26.5 mmol) and $NEt_3$ (3.05 g, 4.1 mL, 30.1 mmol) in dichloromethane (40 mL) and stirred at room temperature for 5 h. After addition of water (30 mL) the phases were separated and the organic phase was washed twice with water (30 mL). The organic phase was dried (anhydrous $Na_2SO_4$) and the solvent was removed under reduced pressure. The crude product was purified by CC (EtOAc; $R_f$ = 0.45) to give **27** (3.27 g, 14.9 mmol, 84%) as a white solid. M.p. 101°C; $^1$H NMR: $\delta$ = 1.21 (t, $J$ = 6.9 Hz, 3H), 2.60 (d, $J$ = 4.8 Hz, 2H), 4.17 (q, $J$ = 7.1 Hz, 2H), 4.27 (d, $J$ = 5.2 Hz, 2H), 8.04 (brs, 1H), 9.76 (brs, 1H), 10.16 ppm (brs, 1H); $^{13}$C NMR: $\delta$ = 14.0 (+), 25.4 (+), 47.6 (−), 61.6 (−), 153.3 ($C_{quat}$), 167.3 ($C_{quat}$), 179.2 ppm ($C_{quat}$); IR (KBr): $\tilde{\nu}$ = 3307, 3177, 3034, 2935, 1720, 1657, 1546, 1414, 1247, 1205, 1043, 963, 739, 615 cm$^{-1}$; MS (EI, 70 eV): $m/z$ (%): 219.3 (100) [$M^+$], 173.2 (10) [$M^+-C_2H_5OH$]; elemental analysis calcd (%) for $C_7H_{13}N_3O_3S$ (219.26): C 38.35, H 5.98, N 19.16; found C 38.15, H 6.48, N 19.23.

**[$N'$-(2-*tert*-Butyloxycarbonylaminoethyl)-$N''$-ethoxycarbonylguanidino]-methyl acetamide (28a)**: Thiourea **27** (41 mg, 0.186 mmol) was added to a solution of 1-Boc-ethylene diamine (44 mg, 0.279 mmol) and $NEt_3$ (37 mg, 50 µL, 0.372 mmol) in DMF (10 mL). Hg$^{II}$ chloride (55 mg, 0.205 mmol) was added in one portion and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in $H_2O$ (15 mL) and acidified with 5% $KHSO_4$ to pH 2–3. The aqueous layer was extracted with EtOAc (3×50 mL). The combined organic layers were dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude product was purified by CC (EtOAc; $R_f$ = 0.15) to yield **28a** (57 mg, 86%) as a white solid. M.p. 112°C; $^1$H NMR: $\delta$ = 1.13 (t, $J$ = 7.1 Hz, 3H), 1.37 (s, 9H), 2.61 (d, $J$ = 5.2 Hz, 2H), 2.97–3.24 (m, 4H), 3.76 (d, $J$ = 5.5 Hz, 2H), 3.91 (q, $J$ = 7.1 Hz, 2H), 6.91 (t, $J$ = 5.5 Hz, 1H), 7.80 (brs, 1H), 8.95 ppm (brs, 1H); IR (KBr): $\tilde{\nu}$ = 3335, 2979, 2935, 1642, 1605, 1535, 1395, 1369, 1286, 1170, 1089, 972, 864, 798, 654 cm$^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{14}H_{27}N_5O_5$: 345.40; found: 346.5 (100) [$M^+$H], 290.4 (35) [$M^+-C_4H_8$], 246.5 (25) [$M^+$H−Boc].

**[2-(*N'*-Methylcarbamoylmethylguanidino)ethyl]carbamic acid allyl ester (31)**. Compound **28b** (77 mg, 0.23 mmol) was dissolved in $H_2O$ (10 mL; Tris-buffer, pH 8.0) and EC3.1.1.1 (10 mg) was added. The reaction mixture was stirred at room temperature for 20 h. The pH value was adjusted to pH 10 with 1N NaOH. The organic layer was extracted with dichloromethane (2×25 mL), the combined organic layers were dried over $Na_2SO_4$ and the solvent was evaporated. The crude product was purified by CC (EtOAc/MeOH 4:1, $R_f$ = 0.35) to yield **31** as a colourless oil (52 mg, 87%). $^1$H NMR: $\delta$ = 2.61 (d, $J$ = 4.9 Hz, 2H), 3.06–3.34 (m, 4H), 3.78–3.87 (m, 2H), 4.47 (d, $J$ = 5.2 Hz, 2H), 5.17 (dd, $J$ = 1.7, 9.3 Hz, 1H), 5.27 (dd, $J$ = 1.7, 16.2 Hz, 1H), 5.80–5.95 (m, 1H), 7.33 (brs, 1H), 7.56–7.73 (m, 2H), 7.89 (brs, 1H), 8.23 ppm (brs, 1H); $^{13}$C NMR: $\delta$ = 25.5 (+), 43.6 (−), 45.2 (−), 59.7 (−), 64.4 (−), 117.0 (−), 133.5 (+), 156.0 ($C_{quat}$), 156.8 ($C_{quat}$), 167.6 ppm ($C_{quat}$); MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{10}H_{19}N_5O_3$ 257.29; found 258.8 (100) [$M^+$H].

**[2-(1,3-Di(*tert*-butoxycarbonyl)-2-methylisothioureido)ethyl]carbamic acid allyl ester (34)**: Compound **32** (250 mg, 0.22 mL, 1.72 mmol), compound **33** (500 mg, 1.72 mmol) and $PPh_3$ (677 mg, 2.58 mmol) were dissolved in THF (25 mL) and DIAD (522 mg, 0.5 mL, 2.58 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated and the crude product was purified by CC (EtOAc/hexane 1:1; $R_f$ = 0.35; EtOAc) to give **34** (552 mg, 78%) as a colourless oil. $^1$H NMR ($CDCl_3$): $\delta$ = 1.40 (s, 9H), 1.48 (s, 9H), 2.38 (s, 3H), 3.30–3.39 (m, 2H), 3.78–3.86 (m, 2H), 4.53 (d, $J$ = 5.2 Hz, 2H), 5.17 (dd, $J$ = 1.7, 9.3 Hz, 1H), 5.27 (dd, $J$ = 1.7 Hz, 16.2 Hz, 1H), 5.83–5.97 (m, 1H), 7.21 ppm (t, $J$ = 5.5 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$ = 6.5 (+), 14.2 ($C_{quat}$), 28.0 (+), 28.1 (+), 28.2 (+), 28.4 (+), 42.4 (−), 42.8 (−), 60.4 ($C_{quat}$), 67.3 (−), 68.3 (−), 80.1 ($C_{quat}$), 83.4 ($C_{quat}$), 131.4 ppm ($C_{quat}$); IR (KBr): $\tilde{\nu}$ = 3256, 3178, 2979, 2912, 1721, 1656, 1511, 1455, 1378, 1166, 987, 845, 756 cm$^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): 418.2 (100) [$M^+$+H], 318.1 (15) [$M^+$+H−Boc], 218.0 (20) [$M^+$+H−2Boc]; elemental analysis calcd (%) for $C_{18}H_{31}N_3O_6S$ (417.19): C 51.78, H 7.48, N 10.06; found C 51.69, H 7.68, N 9.80.

**[$N'$-(2-Allyloxycarbonylaminoethyl)-$N'$,$N''$-di(*tert*-butoxycarbonyl)guanidino]acetic acid methyl ester (35)**: Compound **34** (504 mg, 1.21 mmol) was dissolved in THF (20 mL). After addition of H-Gly-OMe (456 mg, 3.63 mmol) and $NEt_3$ (367 mg, 0.5 mL, 3.63 mmol) the reaction mixture was heated under reflux for 15 h. The solid was filtered off and the solvent was evaporated. The crude product was purified by CC (EtOAc; $R_f$ = 0.65) to yield **35** (403 mg, 75%) as a white solid. M.p. 95°C; $^1$H NMR ($CDCl_3$): $\delta$ = 1.41 (s, 9H), 1.48 (s, 9H), 3.21–3.59 (m, 4H), 3.78 (s, 3H), 4.06 (d, $J$ = 5.2 Hz, 2H), 4.54 (d, $J$ = 5.2 Hz, 2H), 5.19 (dd, $J$ = 1.7, 9.3 Hz, 1H), 5.30 (dd, $J$ = 1.7 Hz, 16.2 Hz, 1H), 5.83–5.97 (m, 1H), 6.87 (t, $J$ = 5.3 Hz, 1H), 8.58 ppm (brs, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$ = 28.0 (+), 28.1 (+), 28.2 (+), 28.4 (+), 40.6 (−), 44.9 (−), 47.5 (−), 52.6 (+), 65.4 (−), 79.9 ($C_{quat}$), 83.4 ($C_{quat}$), 117.4 (−), 117.8 ($C_{quat}$), 133.1 (−), 156.7 ($C_{quat}$), 169.3 ppm ($C_{quat}$); IR (KBr): $\tilde{\nu}$ = 3245, 3009, 2079, 2921, 2856, 1718, 1645, 1434, 1367, 1123, 956 cm$^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd $C_{20}H_{34}N_4O_8$: 458.24; found 481.3 (15) [$M^+$+Na], 459.3 (100) [$M^+$+H], 403.2 (5) [$M^+$+−$C_4H_8$], 359.2 (10) [$M^+$+−Boc].

**[$N'$-(2-Allyloxycarbonylaminoethyl)guanidino]acetic acid methyl ester hydrochloride (36)**: Compound **35** (403 mg, 0.91 mmol) was dissolved in dichloromethane/TFA (4:1; 15 mL) and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dissolved in 1N HCl (10 mL). After lyophylization compound **36** was obtained quantitatively as a white solid. $^1$H NMR: $\delta$ = 3.39–3.68 (m, 4H), 3.87 (s, 3H), 4.11 (d, $J$ = 5.0 Hz, 2H), 4.54 (d, $J$ = 5.2 Hz, 2H), 5.21 (dd, $J$ = 1.7, 9.3 Hz, 1H), 5.32 (dd, $J$ = 1.7 Hz, 16.2 Hz, 1H), 5.83–5.97 (m, 1H), 6.88 (brs, 1H), 8.98 (brs, 2H), 9.34 (brs, 1H), 10.67 ppm (brs, 1H); MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{10}H_{19}N_4O_4$: 259.14; found 259.3 (100) [$M^+$+H].

**(*S*)-2-[$N'$-(2-Allyloxycarbonylaminoethyl)-$N'$,$N''$-di(*tert*-butoxycarbonyl)-guanidino]-3(4-hydroxyphenyl)propionic acid methyl ester (37-Boc)**: Compound **34** (820 mg, 1.96 mmol) and MeO-Tyr-$NH_2$ (574 mg, 2.94 mmol) were dissolved in THF (20 mL) and the reaction mixture was

refluxed for 15 h. The solvent was evaporated and the crude product was purified by CC (EtOAc/hexane 1:1; $R_f = 0.4$) to obtain **37**-Boc (621 mg, 56%) as a pale yellow solid. M.p. 112°C; $[\alpha]_D^{20} = -13.5$ ($c = 0.6$ in MeOH); $^1$H NMR ($CDCl_3$): $\delta = 1.40$ (s, 9H), 1.45 (s, 9H), 2.81–3.50 (m, 6H), 3.78 (s, 3H), 4.05–4.22 (m, 1H), 4.53 (d, $J = 5.2$ Hz, 2H), 5.17 (dd, $J = 1.7$, 9.3 Hz, 1H), 5.28 (dd, $J = 1.7$ Hz, 16.2 Hz, 1H), 5.83–5.97 (m, 1H), 6.78 (d, $J = 8.0$ Hz, 2H), 6.87 (t, $J = 5.3$ Hz, 1H), 6.99 (d, $J = 8.0$ Hz, 2H), 10.12 ppm (brs, 1H); $^{13}$C NMR ($CDCl_3$): $\delta = 28.0$ (+), 28.1 (+), 40.2 (−), 52.6 (+), 65.5 (−), 80.2 ($C_{quat}$), 116.0 ($C_{quat}$), 117.5 (−), 127.1 ($C_{quat}$), 130.4 (+), 133.0 ppm ($C_{quat}$); IR (KBr): $\tilde{\nu} = 3374$, 3076, 2979, 1720, 1615, 1517, 1448, 1368, 1251, 1147, 1069, 994, 929, 840, 773 cm$^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): 565.4 (100) $[M^+ + H]$, 509.3 (20) $[M^+ + H - C_4H_8]$, 465.4 (25) $[M^+ + H - Boc]$; HRMS: $m/z$: calcd for $C_{27}H_{41}N_4O_9$: 565.2874, found 565.2879 ± 0.02 ppm.

**(*S*)-2-[*N*′-(2-Allyloxycarbonylaminoethyl)guanidino]-3(4-hydroxyphenyl)-propionic acid methyl ester hydrochloride (37-H)**: Compound **37**-Boc (621 mg, 1.10 mmol) was dissolved in dichloromethane/TFA 4:1 (15 mL) and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dissolved in 1N HCl (10 mL). After lyophylization compound **37**-H (440 mg, 100%) was obtained as a yellow solid. M.p. 89°C; $^1$H NMR: $\delta = 2.76$–3.21 (m, 6H), 3.78 (s, 3H), 4.05–4.22 (m, 1H), 4.53 (d, $J = 5.2$ Hz, 2H), 5.17 (dd, $J = 1.7$, 9.3 Hz, 1H), 5.28 (dd, $J = 1.7$ Hz, 16.2 Hz, 1H), 5.83–5.97 (m, 1H), 6.75 (d, $J = 8.0$ Hz, 2H), 6.99 (d, $J = 8.0$ Hz, 2H), 7.23 (t, $J = 5.3$ Hz, 1H), 7.61–7.98 (m, 3H), 9.39 ppm (brs, 1H); $^{13}$C NMR: $\delta = 31.2$ ($C_{quat}$), 36.4 (−), 39.2 (−), 39.6 (+), 40.7 (−), 48.5 (+), 52.3 (+), 55.1 ($C_{quat}$), 64.4 (−), 115.1 (+), 117.0 (−), 125.7 ($C_{quat}$), 130.1 (+), 133.5 (+), 155.5 ($C_{quat}$), 170.6 ppm ($C_{quat}$); MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{17}H_{25}N_4O_5Cl$: 400.15; found: 365.1 (100) $[M^+ + H]$.

**(*S*)-2[*N*′-(2-Allyloxycarbonylaminoethyl)-*N*′,*N*″-di(*tert*-butoxycarbonyl)-guanidino]-3-(1*H*-indol-3-yl)propionic acid methyl ester (38-Boc)**: Compound **34** (1.44 g, 3.45 mmol) and MeO-Trp-$NH_2$ (1.32 g, 5.18 mmol) were dissolved in THF (20 mL) and the reaction mixture was heated under reflux for 15 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc/hexane 1:1; $R_f = 0.45$) to yield **38**-Boc (1.26 g, 62%) as a orange oil. $[\alpha]_D^{20} = -24.7$ ($c = 0.6$ in MeOH); $^1$H NMR ($CDCl_3$): $\delta = 1.40$ (s, 9H), 1.46 (s, 9H), 2.81–3.50 (m, 6H), 3.78 (s, 3H), 4.30–4.48 (m, 1H), 4.53 (d, $J = 5.2$ Hz, 2H), 5.18 (dd, $J = 1.7$, 9.3 Hz, 1H), 5.27 (dd, $J = 1.7$ Hz, 16.2 Hz, 1H), 5.81–5.94 (m, 1H), 6.97–7.21 (m, 4H), 7.33 (d, $J = 8.0$ Hz, 1H), 7.55 (d, $J = 8.0$ Hz, 1H), 8.36 (brs, 1H), 10.17 ppm (brs, 1H); $^{13}$C NMR ($CDCl_3$): $\delta = 28.0$ (+), 28.1 (+), 28.2 (+), 28.4 (+), 40.1 (−), 52.4 ($C_{quat}$), 52.6 (−), 65.3 (−), 82.9 ($C_{quat}$), 109.6 ($C_{quat}$), 111.5 (+), 117.3 (−), 119.5 (+), 122.4 (+), 122.9 (+), 123.4 (+), 133.3 ($C_{quat}$), 136.2 ($C_{quat}$), 152.7 ppm ($C_{quat}$); IR (KBr):
$\tilde{\nu} = 3388$, 3059, 2978, 1716, 1618, 1533, 1437, 1368, 1249, 1147, 1064, 1011, 928, 743 cm$^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{29}H_{41}N_5O_8$: 587.29; found: 588.4 (100) $[M^+ + H]$, 532.3 (10) $[M^+ + H - C_4H_8]$, 488.3 (15) $[M^+ + H - Boc]$.

**(*S*)-2-[*N*′-(2-Allyloxycarbonylaminoethyl)guanidino]-3-(1*H*-indol-3-yl)-propionic acid methyl ester hydrochloride (38-H)**: Compound **38**-Boc (1.26 g, 2.14 mmol) was dissolved in dichloromethane/TFA 4:1 (15 mL) and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dissolved in 1N HCl (10 mL). After lyophilization compound **38**-H (906 mg, 2.14 mmol, 100%) was obtained as an orange solid. M.p. 103°C; $^1$H NMR: $\delta = 2.91$–3.34 (m, 6H), 3.78 (s, 3H), 4.30–4.48 (m, 1H), 4.53 (d, $J = 5.2$ Hz, 2H), 5.18 (dd, $J = 1.7$, 9.3 Hz, 1H), 5.27 (dd, $J = 1.7$ Hz, 16.2 Hz, 1H), 5.81–5.94 (m, 1H), 6.97–7.21 (m, 4H), 7.33 (d, $J = 8.0$ Hz, 1H), 7.55 (d, $J = 8.0$ Hz, 1H), 7.59–7.79 (m, 3H), 7.93 (brs, 1H), 9.19 ppm (brs, 1H); IR (KBr): $\tilde{\nu} = 3327$, 1697, 1635, 1527, 1341, 1261, 1151, 932, 745 cm$^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{19}H_{26}N_5O_4Cl$: 423.17; found 388.2 (100) $[M^+ + H]$.

**(*S*)-2-[*N*′-(2-Allyloxycarbonylaminoethyl)-*N*′,*N*″-di(*tert*-butoxycarbonyl)-guanidino]-6-(5-dimethylaminonaphthalene-1-sulfonylamino)hexanoic acid methyl ester (40-Boc)**: Compound **39**-H (397 mg, 1.00 mmol) and compound **34** (626 mg, 1.5 mmol) were dissolved in THF (20 mL). $NEt_3$ (121 mg, 0.166 mL, 1.20 mmol) was added and the reaction mixture was refluxed for 18 h. After filtration the solvent was evaporated and the crude product was purified by CC (EtOAc/hexane 1:1; $R_f = 0.3$) giving **40**-Boc (412 mg, 54%) as a yellow solid. M.p. 103°C; $[\alpha]_D^{20} = +31.0$ ($c = 0.6$ in MeOH); $^1$H NMR ($CDCl_3$): $\delta = 1.31$–1.65 (m, 20H), 1.71–1.83 (m, 2H), 2.83–2.93 (m, 8H), 3.21–3.37 (m, 2H), 3.70 (s, 3H), 4.10–4.21 (m, 1H), 4.53 (d, $J = 5.2$ Hz, 2H), 5.17 (dd, $J = 1.7$, 9.3 Hz, 1H), 5.28 (dd, $J = 1.7$ Hz, 16.2 Hz, 1H), 5.52 (brs, 1H), 5.83–5.97 (m, 1H), 6.87 (brs, 1H), 7.13 (d, $J = 7.7$ Hz, 1H), 7.41–7.53 (m, 2H), 8.11–8.22 (m, 2H), 8.34 (d, $J = 8.0$ Hz, 1H), 9.82 ppm (brs, 1H); $^{13}$C NMR ($CDCl_3$): $\delta = 22.2$ (+), 27.8 (−), 28.1 (+), 28.2 (+), 28.4 (+), 40.4 ($C_{quat}$), 42.7 (−), 45.5 (+), 52.5 (+), 65.5 (−), 68.6 (−), 115.3 (+), 117.4 ($C_{quat}$), 123.3 (+), 128.3 (+), 129.4 (+), 129.8 ($C_{quat}$), 130.2 (+), 152.3 ($C_{quat}$), 157.5 ($C_{quat}$), 171.4 ppm ($C_{quat}$); IR (KBr): $\tilde{\nu} = 3256$, 3112, 2978, 2934, 1722, 1645, 1523, 1411, 1367, 1287, 1188, 1002, 956, 845 cm$^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{36}H_{54}N_6O_{10}S$; 762.36; found: 763.5 (100) $[M^+ + H]$, 663.4 (15) $[M^+ + H - Boc]$.

**(*S*)-2-[*N*′-(2-Allyloxycarbonylaminoethyl)guanidino]-6-(5-dimethylamino-naphthalene-1-sulfonylamino) hexanoic acid methyl ester hydrochloride (40-H)**: Compound **40**-Boc (412 mg, 0.54 mmol) was dissolved in dichloromethane/TFA 4:1 (15 mL) and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dissolved in 1N HCl (10 mL). After lyophilization compound **40**-H (306 mg, 0.54 mmol, 100%) was obtained as a pale yellow solid. M.p. 104°C; $^1$H NMR: $\delta = 1.34$–1.75 (m, 4H), 2.83–2.93 (m, 8H), 3.21–3.37 (m, 2H), 3.72 (s, 3H), 4.10–4.20 (m, 1H), 4.53 (d, $J = 5.2$ Hz, 2H), 5.17 (dd, $J = 1.7$, 9.3 Hz, 1H), 5.28 (dd, $J = 1.7$, 16.2 Hz, 1H), 5.52 (brs, 1H), 5.83–5.97 (m, 1H), 6.89 (brs, 1H), 7.13 (d, $J = 7.7$ Hz, 1H), 7.41–7.53 (m, 4H), 8.11–8.22 (m, 3H), 8.34 (d, $J = 8.0$ Hz, 1H), 8.56 ppm (brs, 1H); IR (KBr): $\tilde{\nu} = 3385$, 2945, 2868, 2787, 1710, 1575, 1508, 1456, 1363, 1319, 1161, 1085, 791, 625, 571 cm$^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{26}H_{39}N_6O_6SCl$: 566.26; found: 563.4 (100) $[M^+ + H]$.

**(*S*)-2-(*N*′,*N*″-Di(*tert*-butoxycarbonyl)-*N*′-{2-[2-(9*H*-fluoren-9-ylmethoxy-carbonylamino)acetylamino]ethyl}guanidino)-3-(4-hydroxyphenyl)-propionic acid methyl ester (45-Boc)**: Fmoc-glycine (110 mg, 0.37 mmol), DIC (47 mg, 0.057 mL, 0.37 mmol) and HOBt (50 mg, 0.37 mmol) were dissolved in dichloromethane (15 mL) and the reaction mixture was stirred at room temperature for 30 min. Compound **37**-H (207 mg, 0.37 mmol), $[Pd(PPh_3)_4]$ (29 mg, 7 mol%) and $Bu_3SnH$ (119 mg, 0.108 mL, 0.41 mmol) were added and the solution was stirred at room temperature for 12 h. The solvent was evaporated and the crude product was purified by CC (EtOAc; $R_f = 0.45$) to obtain **31** (182 mg, 0.25 mmol, 67%) as a colourless oil. $^1$H NMR ($CDCl_3$): $\delta = 1.40$ (s, 9H), 1.47 (s, 9H), 2.81–3.17 (m, 6H), 3.81 (s, 3H), 4.08–4.21 (m, 2H), 4.31–4.43 (m, 4H), 6.02 (brs, 1H), 6.77 (d, $J = 8.2$ Hz, 2H), 7.01 (d, $J = 8.2$ Hz, 2H), 7.11–7.20 (m, 4H), 7.43–7.50 (m, 2H), 7.56–7.63 (m, 2H), 9.01 (brs, 1H), 10.12 ppm (brs, 1H); MS (ESI, dichloromethane/MeOH + 10 mmol L$^{-1}$ $NH_4OAc$): $m/z$ (%): calcd for $C_{40}H_{49}N_5O_{10}$: 759.35, found: 760.4 (100) $[M^+ + H]$.

**Glycine-bridged bis-guanidine 47-Boc**: Compound **45**-Boc (164 mg, 0.21 mmol) was dissolved in 20% piperidine/DMF (10 mL) and the reaction mixture was stirred at room temperature for 1 h. The reaction process was monitored by TLC. The solvent was evaporated and dried under high vacuum. The amine was used without further purification and dissolved in dichloromethane (10 mL), compound **32** (117 mg, 0.21 mmol), DIC (27 mg, 0.033 mL, 0.21 mmol) and HOBt (28 mg, 0.21 mmol) were added and the reaction was stirred at room temperature for 18 h. The solvent was evaporated and the crude product was purified by CC (EtOAc; $R_f = 0.1$) to obtain **33** (38 mg, 17%) as a yellow solid. M.p. 93°C; $^1$H NMR: $\delta = 1.35$ (s, 9H), 1.39 (s, 9H), 1.43 (s, 18H), 2.79–3.43 (m, 12H), 3.78 (s, 3H), 3.85–3.97 (m, 1H), 4.11–4.19 (m, 1H), 4.53 (d, 5.2 Hz, 2H), 4.79–4.88 (m, 2H), 5.17 (dd, $J = 1.7$, 9.3 Hz, 1H), 5.28 (dd, $J = 1.7$ Hz, 16.2 Hz, 1H), 5.83–5.97 (m, 1H), 6.73–7.08 (m, 7H), 7.13–7.19 (m, 2H), 8.23 (brs, 1H), 8.49 (brs, 1H), 9.12 ppm (brs, 1H); $^{13}$C NMR: $\delta = 14.0$ (+), 21.8 (+), 23.2 (+), 24.4 (−), 25.2 (−), 25.7 ($C_{quat}$), 26.2 (−), 27.4 (+), 27.6 (+), 27.7 (+), 27.8 (+), 28.1 (+), 33.2 (+), 40.2 ($C_{quat}$), 40.6 (−), 47.4 (+), 51.8 (+), 54.2 (+), 59.6 ($C_{quat}$), 64.1 (−), 67.7 (−), 109.6

(−), 115.0 (+), 116.8 (−), 119.8 ($C_{quat}$), 119.9 (+), 121.3 (+), 125.3 ($C_{quat}$), 126.6 (+), 127.1 (+), 129.8 (+), 133.5 ($C_{quat}$), 133.6 ($C_{quat}$), 155.8 ($C_{quat}$), 156.1 ($C_{quat}$); IR (KBr): $\tilde{\nu}$ = 3245, 3006, 2988, 2923, 1723, 1634, 1412, 1367, 1234, 1145, 1098, 934, 823 $cm^{-1}$; MS (ESI, dichloromethane/MeOH + 10 mmol $L^{-1}$ $NH_4OAc$): *m*/*z* (%): calcd for $C_{51}H_{75}N_9O_{16}$: 1069.53; found: 1070.6 (100) [$M^+$+H], 970.6 (10) [$M^+$−Boc].

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