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PAPER

Pyrrolic tripodal receptors for carbohydrates. Role of functional groups and binding geometry on carbohydrate recognition†

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The contribution from several H-bonding groups and the impact of geometric requirements on the binding ability of benzene-based tripodal receptors toward carbohydrates have been investigated by measuring the affinity of a set of structures toward octyl β-D-glucopyranoside, selected as a representative monosaccharide. The results reported in the present study demonstrate that a judicious choice of correct geometry and appropriate functional groups is critical to achieve the complementary hydrogen bonding interactions required for an effective carbohydrate recognition.

Introduction

Molecular recognition of carbohydrates is essential in several biological processes, from carbohydrate metabolism and transport, to cell to cell adhesion, cell infection by pathogens, the immune response, and enzyme activity regulation.[1] Since the principles governing these recognition events are yet poorly understood, considerable effort has been directed toward the investigation of saccharide binding using artificial receptors.[2] Over the last two decades, several synthetic receptors have been designed and investigated, showing various levels of recognition towards carbohydrate substrates,[3] some of which exhibited outstanding recognition properties even in water.[4] Some examples from this group are the tripodal receptors **1a–b** (Fig. 1), which bind octyl-β-D-glucopyranoside in chloroform with high affinity and remarkable selectivity.[5] Measurable affinity was also observed in acetonitrile, a significantly more polar solvent, while a modified receptor **2** featuring acetalic substituents was found to possess increased affinity and marked selectivity toward octyl-β-D-mannopyranoside.[6] A common feature of compounds **1–2** is the hexasubstituted benzene scaffold, bearing aminopyrrolic (or iminopyrrolic) units that can interact with carbohydrates through non-covalent forces, mainly hydrogen bonding and CH–π interactions.

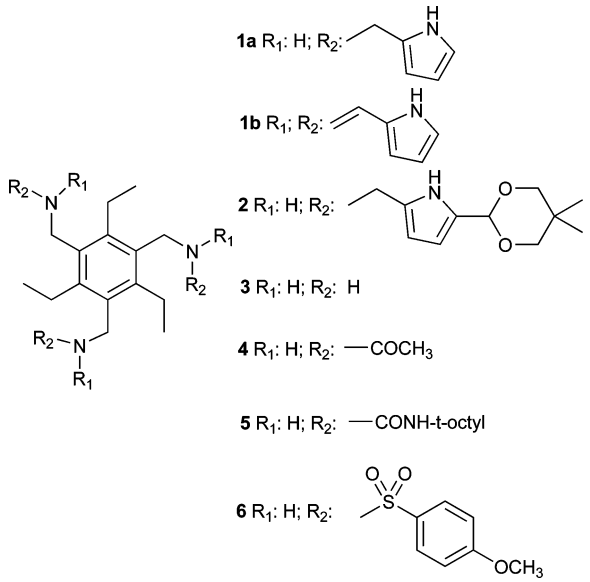

Fig. 1 Structure of the tripodal receptors.

In an effort to expand on recognition properties, we explored alternative binding groups incorporated in the same scaffold. Herein we report on the synthesis of a series of symmetrically substituted tripodal receptors and on their binding properties towards octyl-β-D-glucopyranoside (OctβGlc), selected as a representative monosaccharide. The structural and functional variations described in the present study aimed at the modulation of hydrogen bonding ability, either touching the amine nitrogen or replacing the pyrrolic heterocycle, with the goal of tuning the strength, the directionality and the geometry of interaction within the tripodal architecture.

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† Electronic supplementary information (ESI) available: ^{1}H-NMR spectra of **17a** with OctβGlc in $CDCl_3$ and CD_3CN. ^{1}H-NMR spectra of compounds **6**, **7**, **9**, **10**, **15–18**. See DOI: 10.1039/c0ob00651c

Table 1 Cumulative binding constants (log β_n) for 1 : 1, 2 : 1, 3 : 1 and 1 : 2 host-to-guest complexes of receptors with OctβGlc and corresponding intrinsic median binding concentration BC_{50}^0 (μM) with standard deviation[a]

Entry	log β_{11}	log β_{21}	log β_{31}	log β_{12}	BC_{50}^0/μM
1a[b]	4.61 ± 0.03	7.79 ± 0.06			24 ± 2
1b[b]	5.30 ± 0.05	9.04 ± 0.09			4.8 ± 0.5
3[b]	2.616 ± 0.004				3690 ± 50
4	1.21 ± 0.01				62 000 ± 1000
5[b]	2.67 ± 0.04	4.88 ± 0.06			1970 ± 90
6[b,c]	3.07 ± 0.06	5.86 ± 0.04	7.96 ± 0.17		700 ± 60
7	2.50 ± 0.06			5.11 ± 0.05	1490 ± 80
9	n.d.				n.d.
10	2.61 ± 0.09	4.24 ± 0.13		5.19 ± 0.12	1300 ± 100
15	1.154 ± 0.007				70 000 ± 1000
16	n.d.				n.d.
17b	3.22 ± 0.07	5.46 ± 0.27	8.00 ± 0.20		540 ± 7
18b[b]	3.87 ± 0.01	6.31 ± 0.04			130 ± 4
18c[b]	2.978 ± 0.003				1170 ± 20

[a] Measured by ^{1}H-NMR (400 MHz) from titration experiments at T = 298 K in $CDCl_3$ on 0.8–1.2 mM stock solutions of OctβGlc using receptor concentration up to 25 mM. Binding constants were calculated by simultaneous nonlinear least-square fit of all the available signals shifts. BC_{50}^0 values were calculated from log β_n values using the "BC_{50} Calculator", available for free upon request from one of the authors (S. R.). [b] log β_{dim}: **1a**: 1.07 ± 0.01; **1b**: 0.92 ± 0.02; **3**: 1.83 ± 0.02; **5**: 1.732 ± 0.009; **6**: 2.114 ± 0.006; **18b**: 1.29 ± 0.29; **18c**: 1.74 ± 0.05. [c] log β_{trim}: **6**: 3.69 ± 0.08.

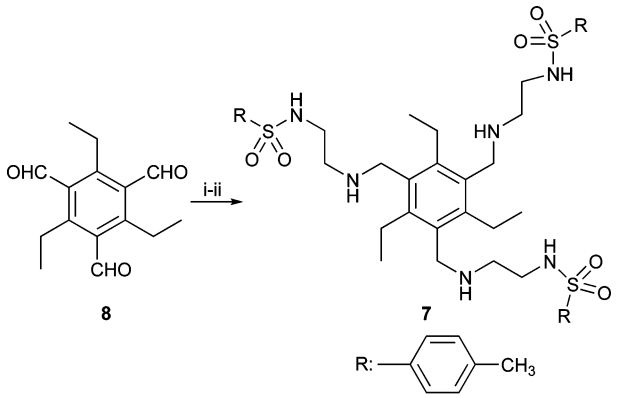
Results and discussion

Sulfonamidic receptors

Since compounds **3–5**,[5,7] bearing protons on nitrogen of different acidity, have shown different binding abilities toward OctβGlc, the effect of increased acidity of the NH function on hydrogen bonding has been investigated by converting the aminic groups of **3** into sulfonamidic moieties.

The sulfonamidic receptor **6** (Fig. 1) was prepared in 81% yield from the parent amine **3** by treatment with 4-methoxybenzenesulfonyl chloride in the presence of triethylamine. The carbohydrate binding ability of **6** was tested in $CDCl_3$ toward OctβGlc by NMR titrations, following a previously established protocol.[7] Since 1 : 1, 2 : 1 and 3 : 1 host-to-guest adducts were detected, in addition to dimerisation of the receptor, the affinity was assessed through the BC_{50}^0 parameter,[5] a generalised affinity descriptor univocally defining the intrinsic binding ability of a receptor in chemical systems involving multiple equilibria. Analogous to the IC_{50} parameter, the lower the BC_{50}^0 value, the higher the affinity. The BC_{50}^0 value for **6**, calculated from cumulative binding constants, is reported in Table 1 together with the values previously obtained for the parent amine **3**,[5] the acetamide **4**[5] and the ureidic derivative **5**.[7]

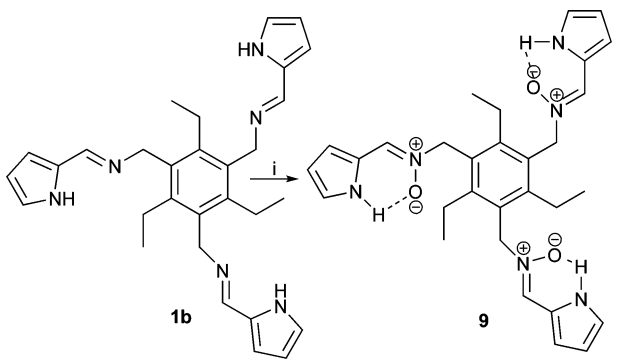
Comparison of the BC_{50}^0 values indicated a somewhat higher affinity of **6** for OctβGlc with respect to receptors **3** and **5**, and much larger than that of **4**. This evidence prompted us to combine the sulfonamidic groups with other H-bonding groups in the tripodal architecture. Homologous replacement of a sulfonamidic NH for the pyrrolic NH of **1a** to give **7** has been achieved in 49% yield by amination of the trialdehyde **8**[8] with *N*-tosylethylenediamine, followed by reduction with $NaBH_4$ (Scheme 1). Disappointingly, this structural variation resulted in a 2-fold decrease in the affinity for OctβGlc with respect to **6** and over a 60-fold drop with respect to **1a**. Results clearly show that increased acidity does not improve the H-bonding ability of the NH function and that the slightly larger affinity of sulfonamide **6** compared to **3** and **5** may rather be ascribed to a more favorable geometry of binding achieved by the sulfonamide NH moiety when located in the benzylic position.



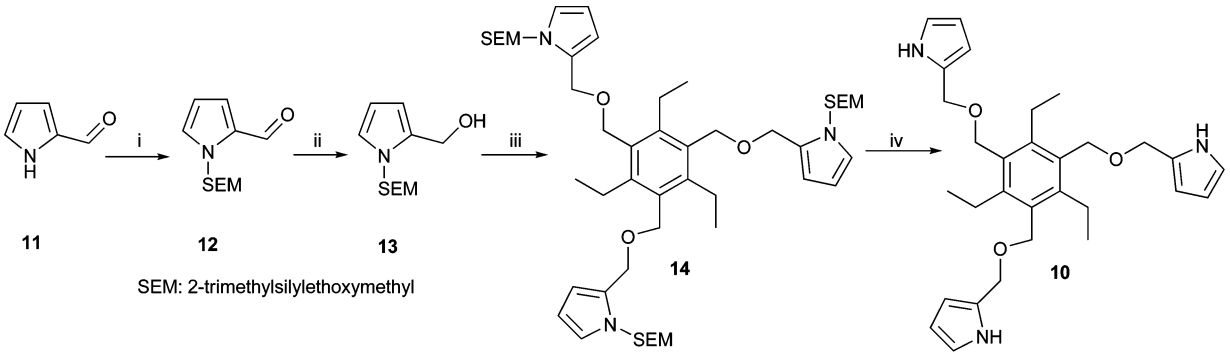
Scheme 1 Synthesis of the sulfonamidic receptor **7**.

Nitronopyrrolic receptor

Nitrones are known to effectively coordinate to metallic Lewis acids,[9] and to interact *via* hydrogen bonding with ureas;[10] moreover, nitrones have been shown to be involved in intramolecular hydrogen bonding, as in the case of *N*-(salicylidene)phenylamine *N*-oxide reported by Brzezinski.[11] Taking advantage of the transformation of an imine into the *N*-oxide by the mild methyltrioxorhenium/urea/hydrogen peroxide catalytic oxidation system,[12] the nitrone derivative **9** has been prepared in 58% yield in one step starting from the imine **1b**[5] (Scheme 2). A strong intramolecular hydrogen bond (as indicated in Scheme 2) has been evidenced from the ^{1}H-NMR spectrum by the downfield shift of the pyrrolic NH to 11.8 ppm. Treatment at room temperature of receptor **9** with increasing amounts of OctβGlc did not induce any shift of the ^{1}H-NMR signals, neither from the sugar nor from the receptor, showing no evidence of interaction with the selected carbohydrate. Most likely, the carbohydrate hydroxyl groups cannot compete with a strategically located pyrrole/nitrone arrangement forming



Scheme 2 Synthesis of the nitronopyrrolic receptor **9**.



i: 2-(trimethylsilyl)ethoxymethylchloride, sodium hydride, DMF, 0 °C, 1h; ii: NaBH4, CH2Cl2/CH3OH 4:1, r.t., 1h; iii: 1,3,5-tris-(bromomethyl)-2,4,6-triethylbenzene, potassium tert-butoxide, DMF, r.t., 1h; iv: tetrabutylammoniumfluoride, 1,2-diaminoethane, DMF, 45 °C, 60h.

Scheme 3 Synthesis of the oxypyrrolic receptor **10**.

a 6-membered H-bonded ring, in order to establish hydrogen bonding interactions.

Oxypyrrolic and pyrrolic receptors

The ether analogue **10** of the amino-pyrrolic receptor **1a** was prepared to ascertain the contribution from the heteroatom located at the benzylic position on the recognition properties of the receptor (Scheme 3). The pyrrolic nitrogen of the aldehyde **11** was protected by reaction with 2-(trimethylsilyl)ethoxymethyl chloride in the presence of sodium hydride in DMF to give **12**,[13] which was reduced with sodium borohydride to the corresponding alcohol **13**. The Williamson etherification of 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene with **13** and potassium *tert*-butoxide in DMF gave **14** in 58% yield over three steps. Deprotection of the pyrrolic groups has been achieved with tetrabutylammonium fluoride and 1,2-diaminoethane[13] to give the ether receptor **10**. The binding ability of **10** toward OctβGlc was evaluated in $CDCl_3$, showing a binding model which included 1 : 1, 2 : 1 and 1 : 2 host-to-guest complexes. The corresponding $BC_{50}{}^0$ value was calculated from cumulative binding constants (see Table 1), and revealed a drop in affinity of over 50-fold with respect to **1a**. The trimethylether derivative **15** of 1,3,5-tris(hydroxymethyl)-2,4,6-triethylbenzene (Fig. 2) was prepared as a reference compound to evaluate the contribution from the pyrrolic group to the binding ability of the ether receptor and from the aminic group to the binding ability of the plain triamine **3**. The 1 : 1 binding constant measured in $CDCl_3$ toward OctβGlc and the corresponding $BC_{50}{}^0$ value calculated for the methyl ether **15** are reported in Table 1, showing a 20-fold decrease in affinity with respect to the amine **3** and over 50-fold with respect to the oxypyrrolic receptor **10**. It is evident that the oxygen atom, which can behave as a hydrogen bonding acceptor exclusively, cannot effectively replace the aminic nitrogen. The results suggest that the amino group takes part in the binding process, and that it may likely participate as a hydrogen bonding donor.

Furthermore, cross-comparison of the affinities of **1a**, **3**, **10**, and **15** shows that the contribution from the pyrrolic group is substantially larger than that of the amine but, when located in the tripodal architecture with the appropriate geometry, both contribute synergetically to the overall binding ability of the receptor, giving an affinity enhancement larger than that expected from their independent contributions. Indeed, although the pyrrolic H-bonding unit has been shown to be essential for the binding properties of the tripodal receptors, its precise location is crucial. This conclusion can be drawn from the results obtained with the receptor **16**, in which a 2-pyrrolyl substituent replaced the amino groups or the oxy- of **3** and **15**, respectively. Receptor **16** has been prepared by incorporating pyrrole rings into the tripodal scaffold through a direct nucleophilic substitution on the alkyl halide.[14] Binding experiments did not show any evidence of interaction of **16** with OctβGlc and the cause for this behaviour may most likely reside in the length of the spacer between the scaffold and the binding group, which prevents the receptor from achieving the correct binding geometry. This result confirms previous observations,[5] showing that elongation of the spacer by one methylene depleted the binding ability of derivatives of **3**.

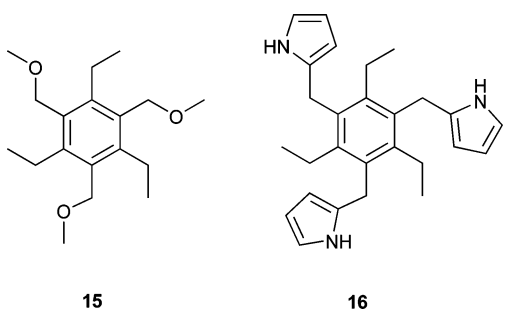
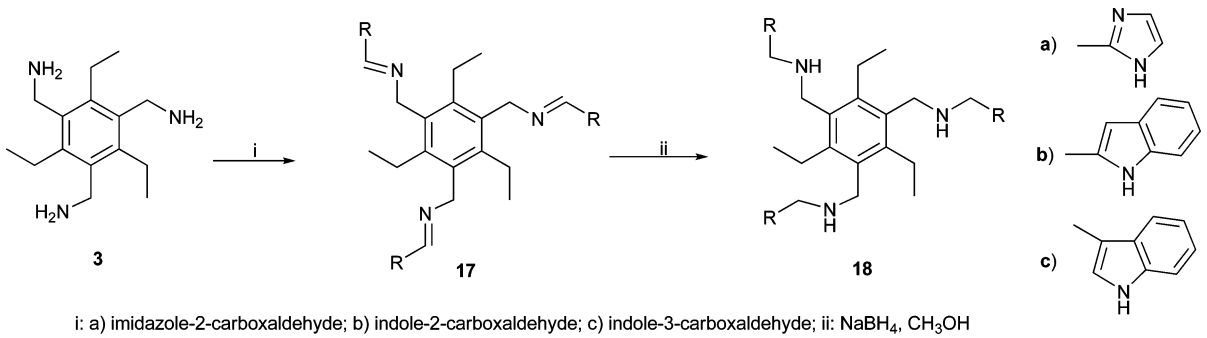


Fig. 2 Structures of methoxymethyl and pyrrolomethyl receptors **15** and **16**.

Imidazolic and indolic receptors

Receptors **17** and **18** (Scheme 4) were designed to explore functional groups alternative to pyrrolic H-bonding donors.[15]

Imidazole features both a pyrrole-like and a pyridine-like nitrogen, thus potentially behaving as a hydrogen bonding donor and acceptor at the same time. This dual character is responsible for the role of imidazole in biological processes, such as those occurring in the active site of enzymes with histidine residues.[16] Receptor **18a** may shed light on the preference of this group to act as a donor or an acceptor of hydrogen bonds, since the two nitrogens occupy structurally equivalent positions in the receptor architecture. **18a** has been synthesized (54%, two steps) by condensation of the



i: a) imidazole-2-carboxaldehyde; b) indole-2-carboxaldehyde; c) indole-3-carboxaldehyde; ii: $NaBH_4$, CH_3OH

Scheme 4 Synthesis of imidazolic and indolic receptors.

parent amine **3** with 2-formylimidazole to give the imine derivative **17a** (71%) which has been reduced to receptor **18a**. The affinity of **18a** for OctβGlc was tentatively investigated by ^{1}H-NMR titration in $CDCl_3$ and CD_3CN but, unfortunately, precipitation of insoluble material during measurements prevented the evaluation of the binding affinity. In addition, the imine precursor **17a** was insoluble in most deuterated solvents (CD_3OD, CD_3CN and $CDCl_3$) whereas good solubility was observed in $(CD_3)_2SO$, in which no evidence of interaction with OctβGlc could be detected. However, when solid **17a** was shaken with a millimolar solution of OctβGlc in $CDCl_3$, the solid partially dissolved and the resulting spectrum showed that 20% of **17a** was present in solution (see Electronic Supplementary Information†). Bound **17a** was still detected (5%) when the experiment was performed in CD_3CN, thus proving that OctβGlc is indeed capable of bringing **17a** into solution by complexation, even though quantitative measurements could not be obtained.

The indolic receptors **17b** and **18b** were obtained by condensation of the amine **3** with indole-2-carboxaldehyde, followed by reduction of the Schiff-base according to the same procedure described for the imidazole derivatives (**18b**, 56% yield, Scheme 4). Likewise, indole-3-carboxaldehyde has been used to give compound **18c** by the same procedure (Scheme 4).[17] The binding abilities of **17b**, **18b** and **18c** toward OctβGlc were tested in $CDCl_3$, where multiple association equilibria were detected in most cases. $BC_{50}{}^0$ values were thus calculated from cumulative binding constants (see Table 1), showing that **18b** is the receptor of highest affinity. It can be appreciated that a drop in affinity of an order of magnitude is observed between **18b** and **18c**, clearly pointing out that connecting the spacer to the 2-position of the pyrrole ring is crucial for achieving an effective binding geometry. On the contrary **18c**, which has the NH located one bond further from the amine, most likely cannot achieve a convergent binding arrangement.

Comparison between the $BC_{50}{}^0$ values of **18b** and **1a** shows that indole is not as effective as pyrrole as a hydrogen bonding donor, the affinity of the former receptor being 5-fold lower than that of the latter. Whether this evidence can be ascribed to electronic factors, steric hindrance, or restricted adaptivity of indole cannot be ascertained from the present data, but the observed loss of affinity is markedly larger when comparing the corresponding iminic receptors **17b** and **1b**, which display an affinity difference of over two orders of magnitude. Considering that the binding ability of the iminopyrrolic receptor **1a** has been shown to rely on the achievement of a chelate H-bonding geometry,[5] the loss of binding ability may be reasonably ascribed to the steric hindrance of the indolic benzene moiety, hampering the achievement of a correct binding conformation and affecting the rigid chelate iminopyrrolic geometry to a larger extent than the flexible aminopyrrolic arrangement.

Conclusions

In summary, a systematic analysis of the structural units constituting the tripodal receptors designed for molecular recognition of carbohydrates has been performed. The results presented highlight that (a) the acidity of sulfonamidic NH groups do not improve H-bonding ability; (b) ethereal oxygen cannot effectively replace amine as an H-bonding group, suggesting that the amine contribution to the recognition process may most likely reside in acting as an H-bonding donor rather than as an H-bonding acceptor; (c) pyrrolic H-bonding units are essential for recognition but a precise location in the architecture is crucial to achieve the correct binding geometry; (d) when the correct geometry is achieved, the aminic and the pyrrolic H-bonding groups exert a synergistic effect, boosting the affinity of the receptor more than their individual contributions; (e) connecting the tripodal scaffold to the 2-position of the indole ring is mandatory for effective recognition, whereas substitution at the 3-position causes a marked drop in binding ability; (f) pyrrole is much more effective than indole as an H-bonding donor when located in the tripodal architecture with the correct geometry, likely because of steric and conformational reasons.

Experimental section

All solvents were of reagent grade quality and purchased commercially. All starting materials were purchased commercially and used without further purification. NMR spectra used for characterization of products and binding experiments were recorded on a Varian Inova 400 instrument. The NMR spectra were referenced to solvent. Mass spectra were recorded on an Agilent Technologies 6110 Quadrupole LC/MS. ESI-MS analysis was performed both in positive or negative ion mode. HRMS were performed on a LTQ-IT-Orbitrap with a spray voltage of 2.10 kV and a resolution of 100 000. C, H and N elemental analysis was performed on a Perkin–Elmer 2400 elemental analyser.

Synthesis of sulfonamidic receptor 6. To a solution of **3**[5] (101 mg, 0.405 mmol) and triethylamine (225 μL, 1.61 mmol) in

CH_2Cl_2 (4 mL), *p*-methoxysulfonyl chloride (253 mg, 1.22 mmol) was added at 0 °C. The reaction mixture was allowed to warm to r.t. and stirred for 1 h. Then it was diluted with CH_2Cl_2 (5 mL) and washed with sat. sol. of NH_4Cl (3 × 5 mL), dried over Na_2SO_4, filtered and concentrated. Purification of the crude product by flash chromatography (CH_2Cl_2–CH_3OH = 20/1, silica gel) gave **6** (130 mg, 0.171 mmol, 42%) as a white solid. M.p. 202–204 °C. Found: C, 57.04; H, 5.93; N, 5.49. Calc. for $C_{36}H_{45}N_3O_9S_3$: C, 56.90; H, 5.97; N, 5.53%; δ_H (400 MHz, $CDCl_3$) 7.84–7.80 (m, 6H, Ph); 7.02–6.99 (m, 6H, Ph); 4.80 (t, J = 4.3 Hz, 3H, NHSO); 3.91 (s, 9H, OCH_3); 3.85 (d, J = 4.3 Hz, 6H, CH_2N); 2.22 (q, J = 7.5 Hz, 6H, CH_2CH_3); 0.80 (t, J = 7.5 Hz, 9H, CH_3). δ_C (100 MHz, $CDCl_3$): 163.2; 144.5; 130.18; 130.14; 129.5; 114.3; 55.7; 40.8; 22.2; 16.1 ppm. MS(ESI): $[M+Na]^+$ = 782.5; $[M+K]^+$ = 798.4.

Synthesis of sulfonamidic receptor 7. To a solution of **8**[8] (218 mg, 0.885 mmol) in CH_3OH (9 mL), *n*-tosylethylenediamine (592 mg, 2.84 mmol) was added at r.t. The solution was stirred for 24 h, then solid $NaBH_4$ (106 mg, 2.79 mmol) was slowly added and evolution of hydrogen observed. After stirring for another 2 h, the mixture was diluted with $CHCl_3$ (50 mL), washed with brine (3 × 5 mL), dried over Na_2SO_4, filtered and concentrated. Purification of the crude by flash chromatography ($CHCl_3$/CH_3OH/NH_3 30% = 20/1/0.15, silica gel) gave **7** (365 mg, 0.434 mmol, 49%) as a white solid. M.p. 58–60 °C. Found: C, 60.32; H, 6.92; N, 10.06. Calc. for $C_{42}H_{60}N_6O_6S_3$: C, 59.97; H, 7.19; N, 9.99%; δ_H (400 MHz, $CDCl_3$, 1.39 mM) 7.73 (d, J = 8.2 Hz, 6H, Ph); 7.30 (d, J = 8.0 Hz, 6H, Ph); 5.04 (br s, 3H, NHSO); 3.61 (s, 6H, NCH_2Ph); 3.02 (m, 6H, CH_2N); 2.81(m, 6H, CH_2N); 2.69 (q, J = 7.4, 6H, CH_2CH_3); 2.41 (s, 9H, CH_3); 1.14 (t, J = 7.4, 9H, CH_2CH_3). δ_C (50 MHz, $CDCl_3$): 143.2; 142.1; 136.9; 133.7; 129.6; 127.1; 48.9; 47.2; 42.7; 22.9; 21.7; 17.1 ppm. MS(ESI): $[M+H]^+$ = 841.00; $[M+Na]^+$ = 863.25.

Synthesis of nitrone receptor 9. To a yellow solution of methyltrioxorhenium (5 mg, 0.020 mmol) and urea hydrogen peroxide (280 mg, 3 mmol) in CH_3OH (3 mL), the solid imine **1b**[5] (150 mg, 0.312 mmol) was added at r.t. Solubilization of the suspension was noted after 10 min, and the reaction mixture was stirred for 1 h more. After solvent removal under reduced pressure, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and the undissolved urea filtered off. Concentration of the solute resulted in a crude mixture, which was purified by flash chromatography ($CHCl_3$/CH_3OH/NH_3 30% = 20/1/0.15, silica gel) to give **9** (95 mg, 0.180 mmol, 58%) as a brown solid. M.p. 144–146 °C. Found: C, 61.76; H, 7.38; N, 14.71. Calc. for $C_{30}H_{36}N_6O_3{\cdot}3H_2O$: C, 61.84; H, 7.27; N, 14.42%; δ_H (400 MHz, $CDCl_3$) 11.86 (br s, 3H, NH); 7.02 (s, 3H, CHN); 6.98–6.95 (m, 3H, Ar); 6.43–6.27 (m, 6H, Ar); 5.19 (s, 6H, CH_2N); 2.86 (q, J = 7.6 Hz, 6H, CH_2CH_3); 1.21 (t, J = 7.5 Hz, 9H, CH_3). δ_C (50 MHz, $CDCl_3$): 147.9; 128.3; 126.3; 124.1; 120.7; 114.5; 110.5; 61.2, 23.8, 15.6 ppm. MS(ESI): $[M+H]^+$ = 529.17; $[M+Na]^+$ = 551.33; $[M+K]^+$ = 567.33.

Synthesis of receptor 10. To a suspension of sodium hydride (1.13 g, 47.1 mmol) in anhydrous DMF (7 mL), pyrrole-2-carboxaldehyde (2.68 g, 28.2 mmol) was added and evolution of hydrogen was observed. The mixture was stirred at r.t. until solubilization and 30 min further. The solution was cooled to 0 °C and 2-(trimethylsilyl)ethoxymethyl chloride (4.71 g, 28.3 mmol) was slowly added. The reaction mixture was stirred for 1 h at 0 °C, then poured into 550 mL of ice-cold $NaHCO_3$ 10% and extracted with CH_2Cl_2 (3 × 200 mL). The organic layers were washed with water (3 × 200 mL), dried over Na_2SO_4, filtered and concentrated to give crude **12** (5.86 g, 26.0 mmol, 92%) as a pale yellow oil. The product was used without further purification in the next reaction. δ_H (200 MHz, $CDCl_3$) 9.59 (s, 1H); 7.15–7.14 (m, 1H); 6.99–6.97 (m, 1H); 6.31–6.29 (m, 1H); 5.71 (s, 2H); 3.58–3.50 (m, 2H); 0.94–0.86 (m, 2H); –0.02–(–0.05) (m, 9H). To a solution of **12** (5.86 g, 26.0 mmol) in CH_2Cl_2 (260 mL), a freshly prepared suspension of $NaBH_4$ (1.97 g, 52.1 mmol) in MeOH (75 mL) was added. The reaction was stirred for 1 h at r.t., poured into water (500 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The organic layers were washed with water (3 × 200 mL), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography (CH_3OH–CH_2Cl_2 = 4/96, silica gel) to give **13** (5.32 g, 23.4 mmol, 90%) as yellow solid. M.p. 36–38 °C. δ_H (200 MHz, $CHCl_3$) 6.78–6.73 (m, 1H); 6.23–6.18 (m, 1H); 6.11–6.05 (m, 1H); 5.29 (s, 2H); 4.62 (d, J = 6 Hz, 2H); 3.55–3.44 (m, 2H); 2.49 (t, J = 6 Hz, 1H); 0.95–0.84 (m, 2H); 0.05–(–0.05) (m, 9H). δ_C (50 MHz, $CDCl_3$) 132.30; 123.00; 110.52; 107.42; 76.30; 65.89; 56.48; 17.98; 1.26 ppm. To a solution of **13** (594 mg, 2.62 mmol) in anhydrous DMF (5.2 mL), potassium *tert*-butoxide (253 mg, 2.25 mmol) was slowly added. To the reaction mixture, 1,3,5-triethyl-2,4,6-tris(bromomethyl)benzene (195 mg, 0.442 mmol) was added with constant stirring over 10 min. The mixture was stirred at r.t. for 1 h, poured into water (70 mL), neutralized with phosphate buffer and extracted with CH_2Cl_2 (3 × 25 mL). The organic layers were combined, washed with water (3 × 50 mL), dried over Na_2SO_4, filtered and concentrated. The crude mixture was purified by flash chromatography (acetone/CH_2Cl_2 = 3/97, silica gel) to give **14** (272 mg, 0.309 mmol, 70%) as a pale yellow glassy solid. δ_H (200 MHz, $CHCl_3$) 6.77–6.70 (m, 3H); 6.24–6.17 (m, 3H); 6.11–6.05 (m, 3H); 5.25 (s, 6H); 4.59 (s, 6H); 4.40 (s, 6H); 3.51–3.39 (m, 6H); 2.61 (q, J = 7.3 Hz, 6H); 1.04 (t, J = 7.3 Hz, 9H); 0.94–0.80 (m, 6H); 0.03–(–0.14) (m, 27H). δ_C (50 MHz, $CDCl_3$) 144.84; 131.75; 128.96; 123.05; 111.57; 107.29; 76.19; 65.50; 65.44; 63.96; 22.66; 17.89; 16.56; 1.18 ppm. To a solution of **14** (676 mg, 0.768 mmol) in DMF (2.5 mL), ethylenediamine (1.03 g, 17.1 mmol) and TBAF (2.18 g, 6.91 mmol) were added. The solution was stirred for 60 h at 45 °C, then poured into water (70 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with water (3 × 50 mL), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography (acetone/CH_2Cl_2 = 10/90, then acetone/CH_2Cl_2 = 20/80, silica gel) to give **10** (55 mg, 0.112 mmol, 15%) as a yellow solid. M.p. 113–114 °C. δ_H (200 MHz, $CDCl_3$) 8.52 (s, 3H); 6.57–6.50 (m, 3H); 6.21–6.14 (m, 3H); 6.14–6.07 (m, 3H); 4.54 (s, 6H); 4.46 (s, 6H); 2.61 (q, J = 7.3, 6H); 1.03 (t, J = 7.3, 9H). δ_C (50 MHz, $CDCl_3$) 144.95; 131.50; 127.75; 118.17; 107.90; 107.59; 64.99; 64.93; 22.22; 16.23 ppm. MS(ESI): $[M+Na]^+$ = 512.4.

Synthesis of receptor 15. To a suspension of 1,3,5-triethyl-2,4,6-tris(bromomethyl)benzene (197 mg, 0.447 mmol) in anhydrous DMF (2.7 mL), sodium methoxide (82 mg, 8.52 mmol) was added at r.t. and the reaction mixture was stirred for 2 h. The mixture was poured into water (30 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were washed with water (3 × 20 mL), dried over Na_2SO_4, filtered and concentrated.

The crude mixture was purified by flash chromatography (ethyl acetate/petroleum ether = 20/80, silica gel) to give **15** (90 mg, 0.306 mmol, 68%) as a white solid. M.p. 84–85 °C. Found C, 73.33; H, 10.27. Calc. for $C_{18}H_{30}O_3$: C, 73.43; H, 10.27; O, 16.30%; δ_H (200 MHz, $CDCl_3$) 4.45 (s, 6H); 3.42 (s, 9H); 2.83 (q, J = 1.75 Hz, 6H); 1.19 (t, J = 1.75 Hz, 9H). δ_C (50 MHz, $CDCl_3$): 144.52; 131.57; 68.37; 57.96; 22.54; 16.26 ppm. MS(ESI): $[M+Na]^+$ = 317.3.

Synthesis of receptor 16. To a suspension of 1,3,5-triethyl-2,4,6-tris(bromomethyl)benzene (1 g, 2.27 mmol) and K_2CO_3 (0.941 g, 6.81 mmol) in CH_2Cl_2 (5 mL) was added pyrrole (15.7 mL, 227 mmol). The whole mixture was allowed to stir for 4 h at room temperature. The reaction was combined with water (50 mL), then the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude mixture was purified by flash column chromatography (ethyl acetate/petroleum ether = 20/80, silica gel) to afford compound **16** (262 mg, 0.66 mmol, 29%) as glassy white solid. δ_H (200 MHz, $CDCl_3$) 7.69 (br s, 3H); 6.62–6.60 (m, 3H); 6.12–6.10 (m, 3H); 5.75–5.73 (m, 3H); 4.04 (s, 6H); 2.62 (q, J = 7.5 Hz, 6H); 1.03 (t, J = 7.5 Hz, 9H). δ_C (50 MHz, $CDCl_3$): 141.31; 133.31; 130.94; 116.18; 108.71; 105.58; 27.93; 23.58; 15.38 ppm. MS(ESI): $[M+H]^+$ = 400.17; $[M+K]^+$ = 438.08.

Synthesis of receptors 17a,b. To a solution of **3**[5] (100 mg, 0.4 mmol) in CH_3OH (3 mL), the corresponding aldehyde (1.2 mmol) was added at r.t. The solution was stirred overnight at r.t., during which a precipitate was formed. The suspension was filtered and washed with fresh CH_3OH, to yield pure imine (**17a**: 71%; **17b**: 73%), as white solid.

17a: M.p. 158–161 °C. Found: C, 64.61; H, 6.98; N, 25.20. Calc. for $C_{27}H_{33}N_9 \cdot H_2O$: C, 64.65; H, 7.03; N, 25.13%; δ_H (400 MHz, DMSO-d6) 8.13 (s, 3H, CH); 7.15 (s, 3H, Ar); 7.02 (s, 3H, Ar); 4.84 (s, 6H, CH_2N); 2.66 (q, J = 7.4 Hz, 6H, CH_2CH_3); 1.12 (t, J = 7.3 Hz, 9H, CH_3). MS(ESI): $[M+H]^+$ = 484.25; $[M+Na]^+$ = 506.42.

17b: M.p. 125–128 °C. Found: C, 77.72; H, 6.87; N, 12.99. Calc. for $C_{42}H_{42}N_6 \cdot H_2O$: C, 77.75; H, 6.84; N, 12.95%; δ_H (200 MHz, $CDCl_3$) 9.15 (br s, 3H, NH-Ind); 8.27 (s, 3H); 7.63–7.59 (m, 3H, Ar); 7.35–7.19 (m, 6H, Ar); 7.12–7.04 (m, 3H, Ar); 6.74 (s, 3H); 4.95 (s, 6H, CH_2N); 2.81 (q, J = 7.3 Hz, 6H, CH_2CH_3); 1.26 (t, J = 7.5 Hz, 9H, CH_3). δ_C (50 MHz, $CDCl_3$): 151.6; 143.1; 136.5; 134.9; 132.5; 127.7; 124.1; 121.4; 119.8; 111.1, 107.5; 56.8; 22.8; 15.6 ppm. MS(ESI): $[M+H]^+$ = 631.6; $[M+Na]^+$ = 653.6; $[M+K]^+$ = 669.6.

Synthesis of receptors 18. To a solution of **3**[5] (100 mg, 0.4 mmol) in CH_3OH (5 mL), the corresponding aldehyde (1.2 mmol) was added at r.t. The solution was stirred overnight at r.t., during which the Schiff base was formed. The reaction mixture was diluted with $CHCl_3$ (20 mL), solid $NaBH_4$ was slowly added and evolution of hydrogen observed. After stirring for another 2 h, the mixture was diluted with $CHCl_3$ (20 mL), washed with brine (3 × 20 mL), dried over Na_2SO_4, filtered and concentrated. Purification of the crude products by flash chromatography (silica gel, $CH_2Cl_2/CH_3OH/NH_3$ 30% = 4/1/0.1 (**18a**), 12/1/0.15 (**18b**) and 5 : 1 : 0.1 (**18c**)) gave **18** (**18a**: 54%, **18b**: 56%, **18c**: 38%) as white solids.

18a: M.p. 116–119 °C. δ_H (200 MHz, $CDCl_3$) 7.09 (s, 6H, CH Ar); 3.83 (s, 6H, CH_2N); 3.29 (s, 6H, CH_2N); 1.87 (q, J = 7.3 Hz, 6H, CH_2CH_3); 0.85 (t, J = 7.3 Hz, 9H, CH_3). HRMS (ESI): calcd. for $[C_{27}H_{39}N_9 + H]^+$ = 490.34012; found 490.34032; delta (ppm): +0.41.

18b: M.p. 152–154 °C. δ_H (400 MHz, $CDCl_3$, 1.6 mM) 8.41 (br s, 3H, NH-Ind); 7.56–7.54 (m, 3H, Ar); 7.31–7.29 (m, 3H, Ar); 7.18–7.07 (m, 6H, Ar); 6.38 (s, 3H, Ar); 4.07 (s, 6H, CH_2N); 3.75 (s, 6H, CH_2N); 2.74 (q, J = 7.3 Hz, 6H, CH_2CH_3); 1.14 (t, J = 7.4 Hz, 9H, CH_3). δ_C (50 MHz, $CDCl_3$, 40 mM): 142.4; 137.3; 136.1; 133.9; 128.4; 121.5; 120.1; 119.6; 110.7; 100.5; 47.6; 47.1; 22.7; 16.9 ppm. MS(ESI): $[M+H]^+$ = 637.08; $[M+Na]^+$ = 659.25. HRMS (ESI): calcd. for $[C_{42}H_{48}N_6 + H]^+$ = 637.40099; found 637.40132; delta (ppm): –0.52.

18c: M.p. 116–118 °C. Found: C, 78.95; H, 7.30; N, 13.00. Calc. for $C_{42}H_{48}N_6$: C, 79.21; H, 7.60; N, 13.20%; δ_H (400 MHz, $CDCl_3$, 10.2 mM) 8.14 (br s, 3H, NH-Ind); 7.64–7.62 (m, 3H, Ar); 7.30–7.28 (m, 3H, Ar); 7.18–7.14 (m, 3H, Ar); 7.10–7.06 (m, 3H, Ar); 7.05–7.04 (m, 3H, Ar); 4.04 (s, 6H, CH_2N); 3.72 (s, 6H, CH_2N); 2.61 (q, J = 7.3 Hz, 6H, CH_2CH_3); 1.00 (t, J = 7.3 Hz, 9H, CH_3). δ_C (100 MHz, $CDCl_3$): 142.1; 136.4; 134.3; 127.2; 122.0; 119.4; 119.0; 111.0; 107.5; 47.3; 45.5; 22.4; 16.7 ppm. MS(ESI): $[M+H]^+$ = 637.6; $[M+Na]^+$ = 659.6; $[M+K]^+$ = 675.6.

Titrations and data analysis. Titrations were performed in 5 mm NMR tubes using Hamilton microsyringes, following a previously described technique.[7] To avoid interference of traces of acid in solution, $CDCl_3$ was additionally treated by eluting through a short column of basic alumina right before use. Mathematical analysis of data and graphics presentation of results were done using the HypNMR 2006[18] computer program from Protonic Software. The program performs simultaneous fit of multiple signals to models involving multiple equilibria, giving binding constants and chemical shifts of individual species. "BC_{50} Calculator", the utility program for computing BC_{50} and $BC_{50}{}^0$, is available for free upon request from one of the authors (S.R.).

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