The first immobilisation of glycoluril-based molecular clips on silica gel and alumina

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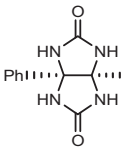
The first example of immobilisation of some glycoluril-based molecular clips, which are good receptors for dihydroxybenzenes, on silica gel and alumina is described. 4,4'-Bis(methoxyphenyl)glycoluril was used as the base scaffold for the synthesis of molecular clips. After demethylation of the methoxy groups, the clip compounds were attached to silica gel and alumina using the linker (3-chloropropyl)trimethoxysilane. The resulting clip-functionalised silica gel and alumina could be applied as a stationary phase in an affinity chromatography technique for the separation and purification of dihydroxybenzenes and the more important biologically active catecholamines.

Keywords: glycoluril, immobilisation, molecular clips, silica gel, alumina

In the late 1980s, a new class of synthetic receptors based on diphenylglycoluril (**1**) was reported in the literature.[1] These receptors, 'molecular clips' as they became known could bind aromatic guests particularly dihydroxybenzenes by sandwiching them between two aromatic surfaces.[2] The origin of the term 'molecular clip' for these molecules is clearly visible from the X-ray structure of compound **2**.[3] The *o*-xylene walls define a tapered cavity and the two fused five-membered rings of the glycoluril form a shallow floor which is electron-rich with two hydrogen-bond receptor sites. ^{1}H NMR studies revealed that clip molecules with their preorganised clefts are excellent receptors for neutral aromatic guests, particularly phenols and dihydroxybenzenes.[4,5] The binding strength of these types of guests within the host can span a wide range of values ($k_a = 0–10^5$ M^{-1}), which vary with simple modifications in either the host or the guests molecule. The binding is the result of three cooperative effects; hydrogen bonding, π–π stacking and a 'cavity effect'.[3] Affinity chromatography is a widely applied separation technique for isolating molecules of biological interest. The technique is based on molecular recognition where one recognition partner is immobilised on a base matrix and soluble target molecules can be retained from a crude mixture. For example, affinity columns with a nucleotide-bonded solid support are used for the separation of various nucleic acids and proteins.[6] Chiral columns with selector-bound solid phase are also used for the separation of enantiomers.[7] Here, we report the first immobilisation of glycoluril-based molecular clips **3a–c** on the surface of silica gel and alumina. The immobilisation led to a new class of materials consisting of rigidly immobilised molecular clips on an inorganic oxide surface, which is expected to find use in the separation and purification of dihydroxybenzenes including catechol, resorcinol and biologically active catecholamines such as dopamine, L-DOPA and adrenaline.



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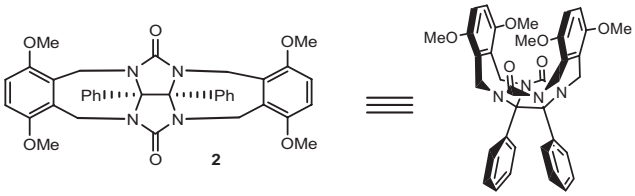

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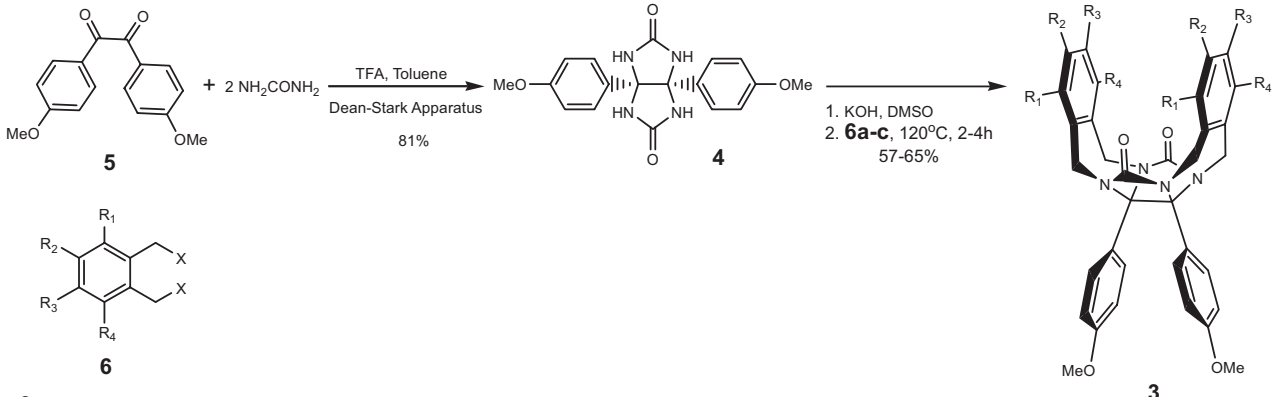
Results and discussion

We have used 4,4'-bis(methoxyphenyl)glycoluril (**4**) as the base skeleton for the clips' subunit. This compound is readily accessible and its methoxy groups can be demethylated to give the more reactive hydroxyl groups for the purpose of immobilising or linking the glycoluril scaffold to a solid phase. So compound **4** was prepared from 4,4'-dimethoxybenzil (**5**)



6a R_1, R_2, R_3, R_4 = H; X = Br
6b R_1, R_4 = H; R_2, R_3 = Me; X = Cl
6c R_1, R_2, R_3, R_4 = Me; X= Cl

3a R_1, R_2, R_3, R_4 = H
3b R_1, R_4 = H; R_2, R_3 = Me
3c R_1, R_2, R_3, R_4 = Me

Scheme 1

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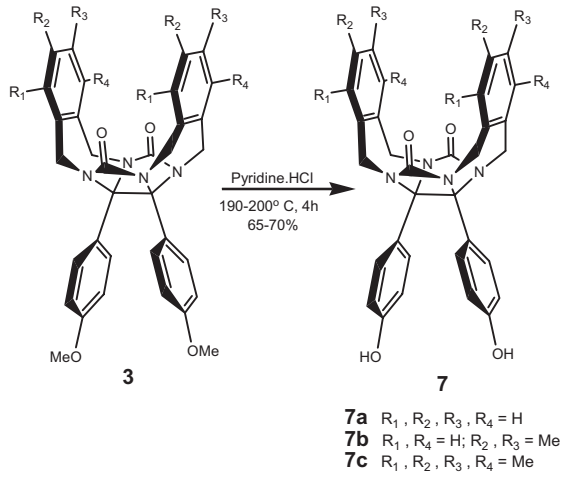


Scheme 2

and urea in TFA and toluene in 81% yield.[8] It was treated with 1,2-bis(halomethyl) compounds **6a–c** and KOH in DMSO to afford the molecular clips **3a–c** in 57–65% yields (Scheme 1). The ^{1}H NMR spectrum of **3b** in $CDCl_3$ displays one pair of well-defined doublets for the CH_2 protons at δ 4.10 and δ 4.71 ppm (J = 16 Hz), two sharp singlets for the methyl and the methoxy groups protons at δ 2.12 and δ 3.70 ppm respectively, and finally the aromatic ring protons as a singlet at δ 7.04 and an AA'XX' system at δ 6.65 and δ 6.98 ppm (J = 8 Hz). It is rational that the binding strength of host and guest molecules is affected by the extent of steric hindrance around the receptor site. Therefore, compounds of type **6a–c** were selected as alkylating agents for the clips in order to exert a variation of steric hindrance around the clip cavity.

Compound **6a** is commercially available, but compound **6b** was prepared by chloromethylation of *o*-xylene using paraformaldehyde and concentrated hydrochloric acid according to a literature procedure.[9] Compound **6c** was prepared in 60% yield from 1,2,3,4,-tetramethylbenzene by the same procedure as described for **6b**. In the next step, compounds **6a–c** were treated with **4** and KOH in DMSO to yield the clip molecules **3a–c** respectively. The hydroxy-containing molecular clips **7a–c** were prepared in 65-70% yields upon reaction of compounds **3a–c** with an excess of fused pyridine hydrochloride at 200°C (Scheme 2). For linking compounds **7a–c** to solid supports (silica gel or alumina), commercially available (3-chloropropyl)trimethoxysilane (**8**) was used as a linker.

The reaction of activated silica gel[10] with **8** in toluene according to a literature procedure afforded (3-chloropropyl)silyl-functionalised silica gel (**9**).[11] Subsequent reaction of **9** with compounds **7a–c** in the presence of potassium iodide and K_2CO_3 in DMSO resulted in immobilisation of **7a–c** on silica gel (**10a-c**) (Scheme 3). The degree of functionalisation of the silica gel was determined by elemental analysis; for example 0.42 mequiv of **7a** was immobilised on 1 g of **10a**. Similarly, the reaction of alumina with **8** as described for **9** afforded (3-chloropropyl)silyl-functionalised alumina (**11**). Subsequent reaction of **11** with compounds **7a–c** led to immobilisation of **7a–c** on alumina (**12a–c**) (Scheme 3). The degree of funtionalisation of the alumina was determined by elemental analysis; for example 0.19 mequiv. of **7a** was immobilised on 1 g of **12a**.

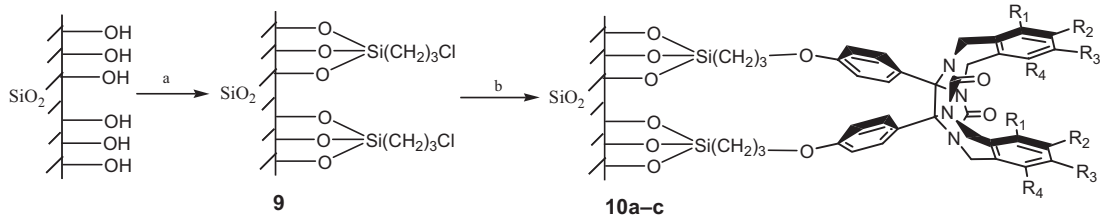
Experimental

Caution: The bis(halomethyl) compounds **6a–c**, especially **6a**, are irritants.

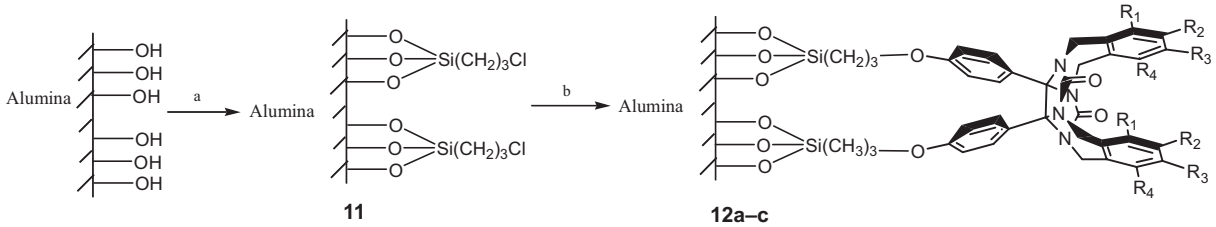
Toluene was distilled from sodium benzophenone ketyl. 4,4'-Dimethoxybenzil (**5**) and 1,2-bis(bromomethyl)benzene (**6a**) were purchased from the Merck company. 1,2,3,4-tetramethylbenzene was purchased from the Aldrich company. 1,2-Bis(chloromethyl)-4,5-dimethylbenzene (**6b**),[9] pyridine hydrochloride[12] and activated silica gel[10] were prepared according to the literature procedures. Melting points were recorded on an electrothermal type 9100 melting point apparatus and are uncorrected. The IR spectra were obtained on a 4300 Shimadzu spectrometer and only noteworthy absorptions are listed. The ^{1}H NMR (100 MHz) spectra were recorded on a Bruker AC 100 spectrometer. Chemical shifts are reported in ppm downfield from TMS as internal standard; coupling constants J are given in Hz. For AA'XX' systems $J^* = J_{23} + J_{25}$. The mass spectra were scanned on a Varian Mat. CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyser.

1,2-Bis(chloromethyl)-3,4,5,6-tetramethylbenzene (**6c**): Prepared in a similar way to **6b** with slight modification as follows: a mixture of 1,2,3,4-tetramethylbenzene (26.8 g, 0.2 mol), paraformaldehyde (18 g, 0.6 mol) and conc. HCl (125 ml) was refluxed with stirring for 10 h. The reaction mixture was cooled and the precipitated solid filtered and washed with water (2 × 50 ml) and cooled hexane (30 ml) and dried *in vacuo*. Yield: 27.7 g (60%); white needles; m.p. = 140–142°C. IR (KBr): 2913, 1439, 1270, 685 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 2.25 (s, 6 H, 2 × CH_3), 2.36 (s, 6 H, 2 × CH_3), 4.80 (s, 4 H, 2 × CH_2).MS (EI): *m/z* (%) = 231 (M^+) Anal. Calc. for $C_{12}H_{16}Cl_2$: C, 89.94; H, 10.06. Found: C, 89.8; H, 10.3.

13b,13c-Di(4-methoxyphenyl)-,7,12,13b,13c,14-hexahydro-5a,6a,12a,13a-tetraazabenzo[5,6]azuleno[2,1,8-ija]benzo



Reaction Condition:(a) **8**, toluene, reflux; (b) **7a–c**, KI, K_2CO_3, DMSO, 120°C, 5day



Reaction Condition:(a) **8**, toluene, reflux; (b) **7a–c**, KI, K_2CO_3, DMSO, 120°C, 5day

Scheme 3

[f]azulene-6,13-dione (**3a**): This compound was synthesised according to a literature procedure using 3.96 g (15 mmol) of compound **6a** and 2.48 g (7 mmol) of compound **4**.[8] M.p. >300°C ^{1}H NMR ($CDCl_3$): δ = 3.70 (s, 6 H, 2 × OCH_3), 4.15 (d, *J* = 16 Hz, 4 H, 4 × CHH), 4.76 (d, J = 16 Hz, 4 H, 4 × CHH), 6.67 (m, *J** = 8 Hz, 4 H, Ar–H), 6.97-7.30 (m, 12 H, Ar–H).

13b,13c-Di(4-methoxyphenyl)-2,3,9,10-tetramethyl-5,7,12,13b,13c,14-hexahydro-5a,6a,12a,13a-tetrazaabenzo[5,6]azuleno[2,1,8-ija]benzo[f]azulene-6,13-dione(**3b**):4,4′-Bis(methoxyphenyl)glycoluril (**4**) (2.48 g, 7 mmol) and freshly ground potassium hydroxide (4.0 g, 70 mmol) in DMSO (50 ml) were heated to 120°C with vigorous stirring for 30 min. Then 1**,**2-bis(chloromethyl)-4,5-dimethylbenzene (**6b**) (3.04 g, 15.0 mmol) was added in one portion and stirring was continued at this temperature for 4 h. On cooling, the reaction mixture was added to water (500 ml) and stirred for 30 min. The resulting light brown precipitate was collected by filtration, washed with water (3 × 400 ml) and acetone (3 × 100 ml), and dried *in vacuo*.Yield: 2.66 g (62%); m.p. >300°C.IR (KBr): 2963, 1709, 1611, 1512, 1458, 1253 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 2.12 (s, 12 H, 4 × CH_3), 3.70 (s, 6 H, 2 × OCH_3), 4.10 (d, *J* = 16 Hz, 4 H, 4 × CHH), 4.71 (d, *J* = 16 Hz, 4 H, 4 × CHH), 6.65 (m, *J** = 8 Hz, 4 H, Ar–H), 6.98 (m, *J** = 8 Hz, 4 H, Ar–H), 7.04 (s, 4 H, Ar–H).MS (EI): *m/z* = 614 (M^+) Anal. Calc. for $C_{38}H_{38}N_4O_4$: C, 74.25; H, 6.23; N, 9.11. Found: C, 74.3; H, 6.5; N, 9.0.

13b,13c-Di(4-methoxyphenyl)-1,2,3,4,8,9,10,11-octa-methyl-,7,12,13b,13c,14-hexahydro-5a,6a,12a,13a-tetraazabenzo[5,6]azuleno[2,1,8-ija]benzo[f]azulene-6,13-dione (**3c**): Prepared as described for **3b** using 3.46 g (15 mmol) of compound **6c** and 2.48 g (7 mmol) of compound **4**. Yield: 2.67 g (57%); mp >300°C. IR (KBr): 2912, 1703, 1610, 1512, 1460, 1253 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 2.15 (s, 12 H, 4 × CH_3), 2.43 (s, 12 H, 4 × CH_3), 3.67 (s, 6 H, 2 × OCH_3), 3.85 (d, *J* = 16 Hz, 4 H, 4 × *CH*H), 5.12 (d, *J* = 16 Hz, 4 H, 4 × *CH*H), 6.62 (m, *J** = 8 Hz, 4 H, Ar–H), 7.02 (m, *J** = 8 Hz, 4 H, Ar–H). MS (EI): *m/z* = 670 (M^+) Anal. Calc. for $C_{42}H_{46}N_4O_4$: C, 75.20; H, 6.91; N, 8.35. Found: C, 75.0; H, 6.9; N, 8.1.

Synthesis of hydroxy-functionalised Clips **7a-c**: *general procedure*
Compound **3** (2.5 mmol) was added to a flask containing pyridine hydrochloride (11.5 g, 0.1 mol) and the mixture was heated at 190–200°C for 4 h. The hot brown syrup was poured into aqueous HCl (5%, 50 ml) and the precipitated solid was filtered and washed with water (3 × 50 ml) and $CHCl_3$ (2 × 25 ml) and dried (*in vacuo* at 80°C) to give compound **7**.

13b,13c-Di(4-hydroxyphenyl)-5,7,12,13b,13c,14-hexahydro-5a,6a,12a,13a-tetraazabenzo [5,6]azuleno[2,1,8-ija]benzo[f]azulene-6,13-dione (**7a**): Prepared according to the general procedure using 1.40 g (2.5 mmol) of compound **3a** and 11.5 g (0.1 mol) of pyridine hydrochloride. Yield: 0.93 g (70%); m.p. >300°C. IR (KBr): 3356 (br), 1682, 1614, 1516, 1464, 1277 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 4.08 (d, *J* = 16 Hz, 4 H, 4 × *CH*H), 4.60 (m, *J** = 16 Hz, 4 H, 4 × *CH*H), 6.58 (m, *J** = 8 Hz, 4 H, Ar–H), 6.83 (d, *J* = 8 Hz, 4 H, Ar–H), 6.97–7.30 (m, 8 H, Ar–H), 9.49 (s, 2 H, 2 × OH). MS (EI): *m/z* = 530 (M^+) Anal. Calc. for $C_{32}H_{26}N_4O_4$: C, 72.44; H, 4.94; N, 10.56. Found: C, 72.6; H, 5.1; N, 10.4.

13b,13c-Di(4-hydroxyphenyl)-2,3,9,10-tetramethyl-5,7,12,13b,13c,14-hexahydro-5a,6a,12a,13a-tetra-azabenzo[5,6]azuleno[2,1,8-ija]benzo[f]azulene-6,13-dione (**7b**): Prepared according to the general procedure using 1.53 g (2.5 mmol) of compound **3b** and 11.5 g (0.1 mol) of pyridine hydrochloride. Yield: 0.98 g (67%); m.p. >300°C. IR (KBr): 3374 (br), 1691, 1615, 1515, 1464, 1279 cm^{-1}. ^{1}H NMR (DMSO- d_6): δ = 2.09 (s, 12 H, 4 × CH_3), 3.99 (d, *J* = 16 Hz, 4 H, 4 × *CH*H), 4.52 (d, *J* = 16 Hz, 4 H, 4 × *CH*H), 6.56 (m, *J** = 8 Hz, 4 H, Ar–H), 6.81 (m, *J** = 8 Hz, 4 H, Ar–H), 6.94 (s, 4 H, Ar–H), 9.47 (s, 2 H, 2 × OH). MS (EI): *m/z* = 586 (M^+) Anal. Calc. for $C_{36}H_{34}N_4O_4$: C, 73.70; H, 5.84; N, 9.55. Found: C, 73.95; H, 6.0; N, 9.7.

13b,13c-Di(4-hydroxyphenyl)-1,2,3,4,8,9,10,11-octamethyl-5,7,12,13b,13c,14-hexahydro-5a,6a,12a,13a-tetraazabenzo[5,6]azuleno[2,1,8-ija]benzo[f]azulene-6,13-dione (**7c**): Prepared according to the general procedure using 1.67 g (2.5 mmol) of compound **3c** and 11.5 g (0.1 mol) of pyridine hydrochloride. Yield: 1.04 g (65%); m.p. >300°C. IR (KBr): 3381 (br), 1685, 1614, 1515, 1470, 1278 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 2.07 (s, 12 H, 4 × CH_3), 2.34 (s, 12 H, 4 × CH_3), 3.73 (d, *J* = 16 Hz, 4 H, 4 × *CH*H), 4.94 (d, *J* = 16 Hz, 4 H, 4 × *CH*H), 6.55 (m, *J** = 8 Hz, 4 H, Ar–H), 6.90 (m, *J** = 8 Hz, 4 H, Ar–H), 9.42 (s, 2 H, 2 × OH). MS (EI): *m/z* = 642 (M^+) Anal. Calc. for $C_{40}H_{42}N_4O_4$: C, 74.74; H, 6.59; N, 8.72. Found: C, 74.5; H, 6.8; N, 8.55.

(3-Chloropropyl)silyl-functionalised silica gel (**9**): The procedure previously described for **9**[11] was followed: 10 g of activated silica gel (Fluka No. 60741) and 3.0 g of **8** in 50 ml anhydrous toluene yielded 10.5 g chloropropyl silica. By elemental analysis for chlorine (3.38%) the loading was found to be 0.95 mequiv g^{-1}.

Preparation molecular clips immobilised on silica gel (**10a–c**); *general procedure*
A mixture of **9** (1 g), K_2CO_3 (0.26 g), potassium iodide (0.32 g) and **7** (0.47 mmol) in 10 ml DMSO was stirred and heated in a bath at 120°C for 5 days. The functionalised silica 10 was filtered and washed successively with DMSO (2 × 5 ml), H_2O (2 × 5 ml) and finally MeOH (2 × 5 ml) and then dried in vacuo at 70°C for 24 h. Elemental analysis of nitrogen for **10a–c** indicated a loading of 0.42 mequiv, 0.45 mequiv and 0.40 mequiv **7a–c** g^{-1} of **10a–c** respectively.

(3-Chloropropyl)silyl-functionalised alumina (**11**): Prepared according to the literature procedure from dry (dried at 100°C at 2 mmHg; 5 h) alumina (Merck No. 101067) (10 g) and **8** (1.95 ml, 10 mmol) in refluxing toluene.[13] Elemental analysis showed that 0.45 mmol of **8** was immobilised on 1 g of **11**.

Preparation molecular clips immobilised on alumina (**12a–c**); *general procedure*
Prepared in a manner similar to that described for the preparation of **10a–c**. Elemental analysis for nitrogen of **12a–c** showed a loading of 0.19 mequiv, 0.20 mequiv and 0.17 mequiv **7a–c** g^{-1} of **12a–c** respectively.

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