Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 3889–3892

TETRAHEDRON LETTERS

On-beads screening of solid-attached diketopiperazines for calix[5]arene-based receptor

Takeharu Haino, Hisashi Mitsuhashi and Yoshimasa Fukazawa*

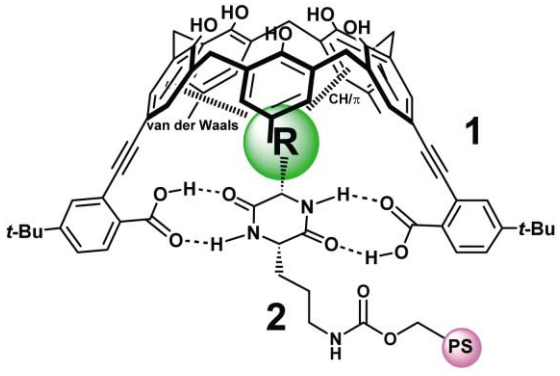
Department of Chemistry, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, 739-8526, Japan

Received 3 March 2003; revised 26 March 2003; accepted 31 March 2003

Abstract—On-beads binding studies of solid-bound DKPs to the calix[5]arene-based receptor were achieved. The results of the binding abilities of the DKPs to the receptor are quite consistent with the binding constants determined by the solution titration studies.

In the past few decades, supramolecular chemistry has grown into a fascinating research area, and gives us important insights on intermolecular non-covalent interactions.[1] Despite the tremendous progress in the design and synthesis of receptor molecules with predicted binding properties, it remains a difficult task to find an efficient receptor for a particular guest. The approach finding the receptor includes three stages: design, synthesis, and screening. These stages are obviously time-consuming. Therefore, recent efforts focused on the more efficient way of the finding process.[2] Combinatorial chemistry is so far one of the most promising approaches to accelerate the discovery of the molecule having desired functions. Solid-phase synthesis has been a major tool to provide the diverse compound libraries. It gives a quick generation of the receptor library capable of binding certain desired guests.[3]

We have been working on developing the artificial receptors based on calixarenes capable of binding neutral guests with hydrogen bonds.[4] Calix[5]arene-based receptor **1** shows effective binding to the diketopiperazines (DKPs) having a variety of alkyl substituents.[5] Non-directional forces between the alkyl substituents **R** and the receptor cavity play a key role on the guest-selection. It is time-consuming to determine the binding constants of each DKP to the receptor. In order to find more active DKPs, we applied the solid-phase combinatorial chemistry for the evaluation of the guest binding ability to **1**.

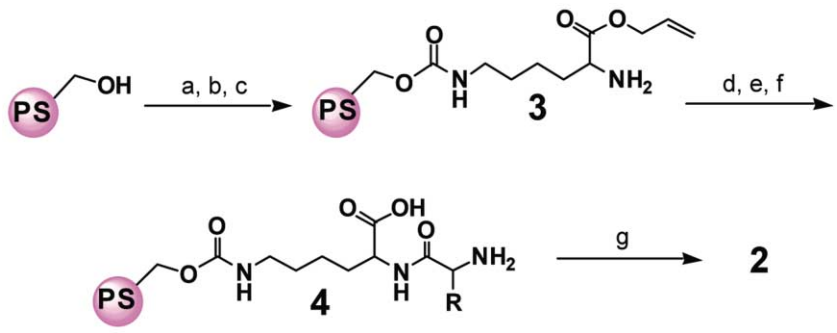


Screening techniques for active compounds are of crucial importance for the success of the combinatorial concept. Time-consuming but more steady way is the release of the guest from solid support and direct estimation of the binding constants by titration. The direct screening of resin-bound libraries provides very fast assay.[6] Direct on-beads assay, however, has some drawbacks; major one is the low accessibility of the receptor to the immobilized guests. Steric hindrance of the polymer supports of the beads hampered the easy access of the receptor.[7] It can be improved to some extent by the use of flexible linkers. Argo–Wang resins were hence selected as a polymer support.

The solid-phase synthesis was applied onto the construction of the DKP library in parallel fashion (Scheme 1). Parallel synthesizer, Quest210, was used for the synthesis. Fmoc protected L-lysine allyl ester was attached to Argo–Wang resins through the carbamate linkage, which is readily cleavable with acid catalysis. Deprotection of Fmoc group with 10% piperidine gave

* Corresponding author. Tel.: +81 824 24 7427; fax: +81 824 24 0724; e-mail: fukazawa@sci.hiroshima-u.ac.jp

0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)00825-6



Scheme 1. *Reagents and conditions*: (a) 4-$O_2NC_6H_4O_2CCl$, NMM/CH_2Cl_2 12 h; (b) Fmoc-Lys-OAllyl, DIEA/DMF 16 h; (c) 10% piperidine/DMF 30 min; (d) Fmoc-AA-OH, DIC, HOBT/DMF 10 h; (e) $Pd(PPh_3)_4$, Ph_3SiH/CH_2Cl_2 2 h; (f) 10% piperidine/DMF 30 min; (g) DIC, C_6F_5OH, DMAP/THF 10 h.

lysine attached resin **3**. Fmoc protected seventeen different amino acids were introduced onto the resulted resin **3**. Deprotection of allyl ester under palladium catalysis followed by a removal of Fmoc group gave amino acids **4**. Although the usual reagents (EDCI-HOBT, DIC-HOBT, etc.) for the cyclization reaction did not work nicely, the conditions with DIC, pentafluorophenol, and DMAP gave desired DKPs $\mathbf{2}_{a1\text{-}a7}$, $\mathbf{2}_{a9\text{-}b7}$, and $\mathbf{2}_{c8}$ in good purity. DKPs $\mathbf{2}_{c1\text{-}c7}$ were prepared from $\mathbf{2}_{a1}$. Deprotection of TBS group with TBAF in THF for 4 h gave $\mathbf{2}_{a8}$. Introduction of ester function to $\mathbf{2}_{a8}$ was carried out with DIC, DMAP, and the corresponding carboxylic acids in DMF for 12 h. Finally, 25 members of DKPs **2**, listed in Figure 1, were synthesized on solid support.

Binding of **1** to the guest on the solid supports can be monitored through the decrease of absorbance of free receptor **1** in solution.[6] We used this tactic to characterize the binding abilities of receptor **1** to the DKPs on solid-support. When DKP-bound resin $\mathbf{2}_{a4}$ were mixed in the standard solution (4.6×10^{-5} mol/L) of the receptor in $CHCl_3$, the resins separated on the top of the solution. The amount of the receptor bound to the on-beads guest can be monitored by the decrease of the receptor concentration. By adding the polymer bound DKP $\mathbf{2}_{a4}$, a linear decrease was observed in the absorbance at $\lambda = 275$ nm of the receptor (Fig. 2).

A plot of the absorption changes versus amount of the added resin $\mathbf{2}_{a4}$ produced the straight line (Fig. 3). Non selective adsorption of the receptor onto the polymer resin may interfere the estimation of the host–guest selective binding. In order to evaluate the simple adsorption of the receptor, the control experiment was carried out. When Wang resin itself was added to the receptor solution, no absorbance change was observed. The adsorption of the receptor is thus negligible in this case. The slopes for other DKPs were determined in the same manner and shown in Figure 4. The slopes correspond to the relative binding abilities of the DKPs.

We have known the binding abilities of the DKPs **5**–**9** (Fig. 5) in solution.[5] In order to know the reliability of the slopes of the polymer-bound guests, some of them were compared to the known binding constants. The

$\mathbf{2}_{a1}$: R=	$TBSOCH_2$	$\mathbf{2}_{b1}$: R=	*p*-*t*-$BuOC_6H_4CH_2$
$\mathbf{2}_{a2}$	$CyCH_2$	$\mathbf{2}_{b2}$	*p*-$NO_2C_6H_4CH_2$
$\mathbf{2}_{a3}$	*t*-Bu	$\mathbf{2}_{b3}$	*p*-$MeOC_6H_4CH_2$
$\mathbf{2}_{a4}$	*i*-Bu	$\mathbf{2}_{b4}$	*p*-$ClC_6H_4CH_2$
$\mathbf{2}_{a5}$	*n*-Bu	$\mathbf{2}_{b5}$	*p*-$FC_6H_4CH_2$
$\mathbf{2}_{a6}$	*n*-Pr	$\mathbf{2}_{b6}$	$PhCH_2$
$\mathbf{2}_{a7}$	Et	$\mathbf{2}_{b7}$	2-thienylCH_2
$\mathbf{2}_{a8}$	$HOCH_2$	$\mathbf{2}_{c1}$: R=	3, 5-$Br_2C_6H_3COOCH_2$
$\mathbf{2}_{a9}$	Me	$\mathbf{2}_{c2}$	3, 5-$Me_2C_6H_3COOCH_2$
$\mathbf{2}_{a10}$	H	$\mathbf{2}_{c3}$	*p*-$IC_6H_4COOCH_2$
		$\mathbf{2}_{c4}$	$MeCH{=}C(Me)COOCH_2$
		$\mathbf{2}_{c5}$	$MeCHClCOOCH_2$
		$\mathbf{2}_{c6}$	*i*-$PrCOOCH_2$
		$\mathbf{2}_{c7}$	$EtCOOCH_2$
		$\mathbf{2}_{c8}$	$AcOCH_2$

Figure 1. Solid-bound DKPs **2**.

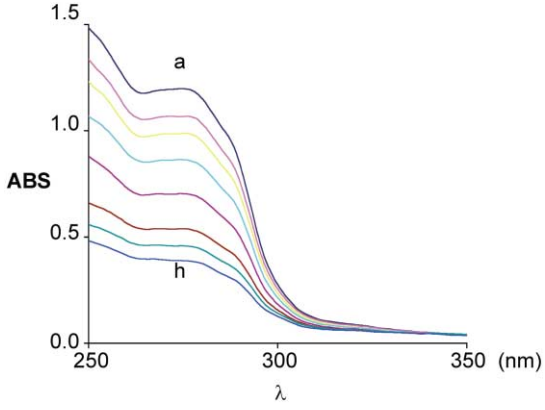


Figure 2. The absorption spectra of receptor **1** (4.6×10^{-5} mol/L) in $CHCl_3$ upon the addition of the polymer-bound DKP $\mathbf{2}_{a4}$: (a) 0.0, (b) 19.3, (c) 38.6, (d) 57.9, (e) 77.2, (f) 96.5, (g) 115.8, (h) 135.1 equiv.

plots of the binding constants versus the slopes gave a straight line with a good correlation ($R^2 = 0.96$) (Fig. 6). It indicates that the slopes represent the accurate relative binding abilities of the solid-bound DKPs. The on-beads assay is thus reliable for the evaluation of the relative binding abilities of the receptor.

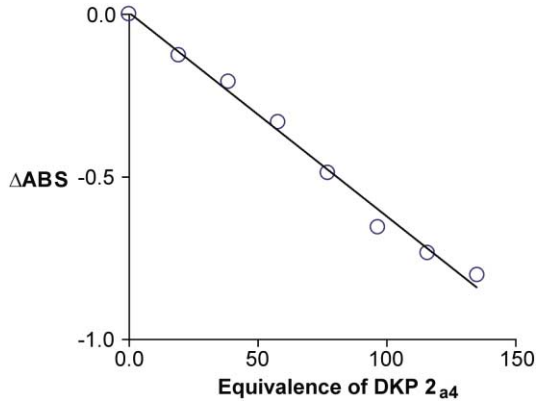


Figure 3. The plot of absorption changes versus the amount of DKP $\mathbf{2_{a4}}$.

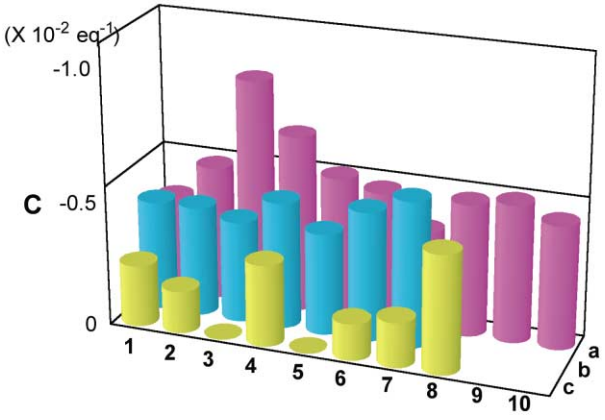
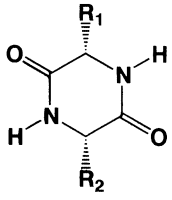


Figure 4. Slopes (C) of the plots between the absorption changes versus the amount of solid-bound DKPs.



5: R_1, R_2= ***t*-Bu**
6: ***i*-Bu**
7: **$PhCH_2$**
8: **Me**
9: **$TBSOCH_2$**
10: R_1= ***p*-*t*-BuOPhCH_2**
R_2= **$(CH_2)_4NHCOOCH_2Ph$**
11: R_1= **$AcOCH_2$**
R_2= **$(CH_2)_4NHCOOCH_2Ph$**

Figure 5. Reference compounds **5**–**11**.

On the basis of our previous results, the size of the α-substituents on the DKPs plays an important role in the guest selectivity.[5b] In the series of DKP $\mathbf{2_a}$, the alkyl substituents which occupies around 70% of the receptor cavity[5b] (100 Å^3) produced the good stabilization of the complex (Table 1). This result is consistent with our previous results.[5b]

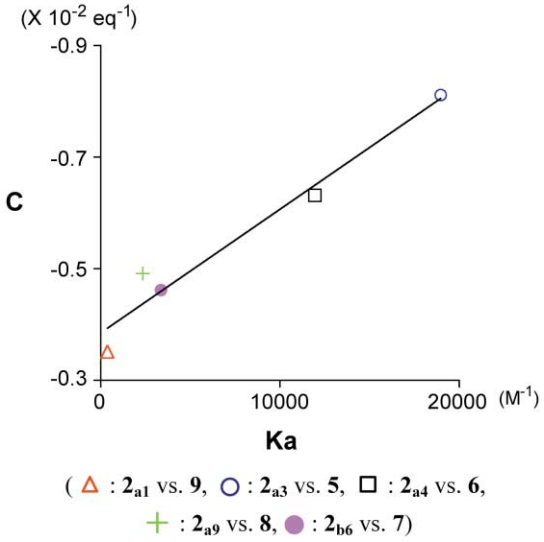


Figure 6. The correlation plot of slopes (**C**) versus binding constants (**Ka**) of DKPs.

All of the DKPs $\mathbf{2_{b1}}$–$\mathbf{2_{b7}}$ having CH_2-phenyl derivative and its thiophene analogue as the α-substituent showed rather high binding ability though the volumes of the substituents are much larger than that of $\mathbf{2_{a3}}$. Although the full enclosure of the bulky α-substituent by the receptor cavity wall cannot be attained, molecular mechanics calculations suggested that a good deal of contact between the phenyl ring of the guest and the cavity wall should be a reasonable explanation of the effective binding (Fig. 7).

A series of DKPs $\mathbf{2_c}$ having ester function do not show effective binding to the receptor except $\mathbf{2_{c8}}$[9] although DKPs $\mathbf{2_{c5\text{-}8}}$ have similar volumes of the α-substituents to those of DKPs $\mathbf{2_{a2\text{-}5}}$. When the DKPs bind to the receptor, O=C–O part of the substituent situates within the π-basic cavity. Since the polarizability of oxygen atoms in the ester functionality is smaller than aliphatic $–CH_2–$ or aromatic –CH–, van der Waals interaction between the O=C–O part of the ester group and the receptor cavity wall is much smaller than that of the alkyl group of a similar volume. Some of the bulky esters have no binding tendency. Some electrostatic interactions between the ester oxygens and the electron rich aromatic rings might produce unfavorable intermolecular interaction.

We have presented the solid-phase synthesis of the DKPs and on-beads assay to the calix[5]arene receptor. Our screening method of the binding abilities to the receptor is reliable for the hydrogen binding host–guest complexes.

Acknowledgements

We are grateful to Argonaut Technologies for their kind help. This work was supported by a Grant-in-Aid

Table 1. Volumes[a] (Å^3) of the α-substituents on the DKPs

$TBSOCH_2$	159	*t*-$BuOPhCH_2$	169	3,5-$Br_2PhCOOCH_2$	172
$CyCH_2$	106	*p*-NO_2PhCH_2	123	3,5-$Me_2PhCOOCH_2$	156
t-Bu	69	*p*-$MeOPhCH_2$	119	*p*-$IPhCOOCH_2$	124
i-Bu	69	*p*-$ClPhCH_2$	108	$MeCH{=}C(Me)COOCH_2$	113
n-Bu	69	*p*-$FPhCH_2$	97	$MeCHClCOOCH_2$	102
n-Pr	54	$PhCH_2$	93	*i*-$PrCOOCH_2$	102
Et	40	2-$ThienylCH_2$	90	$EtCOOCH_2$	86
$HOCH_2$	33			$AcOCH_2$	70
Me	23				
H	4				

[a] The volumes were calculated by Grasp program.[8]

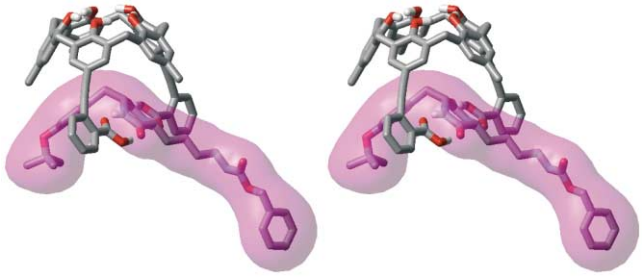


Figure 7. The stereo plot of the calculated structure of the host–guest complex with DKP **10**.[10]

(14654114) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. (a) Schneider, H.-J.; Dürr, H. *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; VCH: Weinheim, 1991; (b) Lehn, J.-M. *Supramolecular Chemistry*; VCH: Weinheim, 1995.
2. (a) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155–163; (b) Ganesan, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2828–2831.
3. (a) Jensen, K. B.; Braxmeier, T. M.; Demarcus, M.; Frey, J. G.; Kilburn, J. D. *Chem. Eur. J.* **2002**, 1300–1309; (b) Shneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542–543; (c) Löwik, D. W. P. M.; Weingarten, M. D.; Broekema, M.; Brouwer, A. J.; Still, W. C.; Liskamp, R. M. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1846–1850.
4. (a) Haino, T.; Matsumura, K.; Harano, T.; Yamada, K.; Saijyo, Y.; Fukazawa, Y. *Tetrahedron* **1998**, *54*, 12185–12196; (b) Haino, T.; Katsutani, Y.; Akii, H.; Fukazawa, Y. *Tetrahedron Lett.* **1998**, *39*, 8133–8136; (c) Haino, T.; Akii, H.; Fukazawa, Y. *Synlett* **1998**, 1016–1018; (d) Haino, T.; Yamada, K.; Fukazawa, Y. *Synlett* **1997**, 673–674; (e) Haino, T.; Harano, T.; Matsumura, K.; Fukazawa, Y. *Tetrahedron Lett.* **1995**, *36*, 5793–5796.
5. (a) Haino, T.; Nitta, K.; Saijo, Y.; Matsumura, K.; Hirakata, M.; Fukazawa, Y. *Tetrahedron Lett.* **1999**, *40*, 6301–6304; (b) Haino, T.; Nitta, K.; Fukazawa, Y. *Tetrahedron Lett.* **2000**, *41*, 4139–4142.
6. (a) Rafai Far, A.; Cho, Y. L.; Rang, A.; Rudkevich, D. M.; Rebek, J., Jr. *Tetrahedron* **2002**, *58*, 741–755; (b) Rafai Far, A.; Rudkevich, D. M.; Haino, T.; Rebek, J., Jr. *Org. Lett.* **2000**, *2*, 3465–3468.
7. Yan, B. *Acc. Chem. Res.* **1998**, *31*, 621–630.
8. Nicholls, A.; Sharp, K. A.; Honig, B. *Proteins* **1991**, *11*, 281.
9. Molecular mechanics calculation showed the effective CH-π interaction between the acetyl methyl and the cavity wall of the model complex of **1** and DKP **11**.
10. Molecular mechanics calculation of the host–guest complex was carried out by MacroModel V. 6.5 using MMFF with GB/SA chloroform solvation model: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comp. Chem.* **1990**, *11*, 440–467.