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Exploiting Site–Site Interactions on Solid Support to Generate Dimeric Molecules

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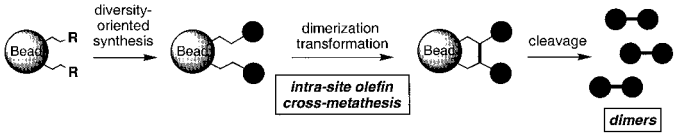
ABSTRACT



"Intra-site" olefin cross-metathesis on solid support leads to nearly quantitative yields of dimeric molecules.

Many signal transduction pathways are initiated by the binding of bivalent ligands, usually proteins, to their receptors on the cell surface.[1] In some cases, homodimerization of these receptors is sufficient to generate an intracellular response.[2,3] The concept of promoting protein−protein interaction as a means of regulation has inspired the design of small molecules to inducibly associate proteins.[2−4] To meet this goal rapidly and for a wide range of target proteins, we have focused on a general method to prepare C_2-symmetric small molecules on solid support. Recent approaches to the solid-phase synthesis of homo- and heterodimeric compounds have involved a bifunctional scaffold bound to the solid support, which is either elaborated in parallel (Figure 1a)[5] or orthogonally protected and functionalized in a stepwise fashion (Figure 1b), respectively.[6,7] A different approach to the synthesis of homodimeric molecules on solid support involves the "intra-site" reaction between two reactive monomers bound to the same polymer bead. Cleavage from the polymeric support then yields the fully symmetrical dimers in solution (Figure 1c).

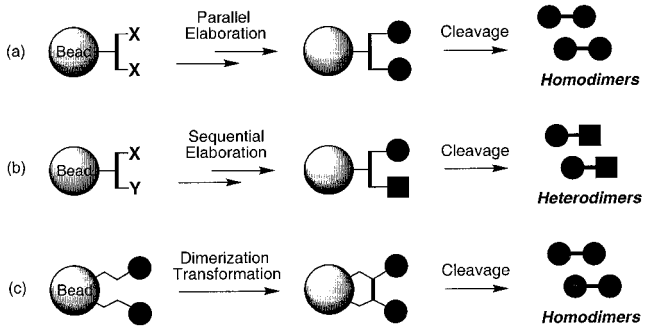


Figure 1. Three synthetic strategies for the synthesis of homo- and heterodimeric molecules on solid support.

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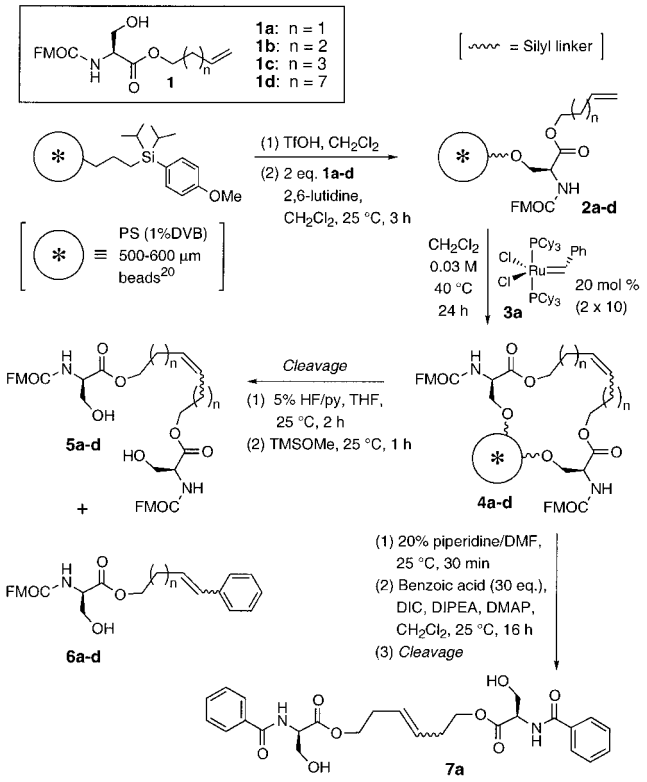
Olefin cross-metathesis[8,9] has been used previously in the efficient solution-phase synthesis of individual dimeric molecules[10] and libraries thereof.[11] While olefin metathesis has been performed previously on solid support,[8,12] there have been few reports of the intentional solid-phase synthesis of symmetrical molecules by cross-metathesis.[13] Herein, we report the successful synthesis of symmetric molecules by an intra-site cross-metathesis reaction on individual polymer beads.[14] Notably, this transformation represents an efficient, "one-pot" procedure to synthesize homodimers of virtually any molecule amenable to our solid-phase platform.

Intra-site reactions, or site−site interactions, have been reported with some frequency since the invention of solid-phase chemistry by Merrifield in 1963.[15] At the outset, it was believed that substrates bound to polymeric supports were effectively "site-isolated" from each other, inhibiting any cross-reactivity.[16] However, it was quickly discovered that site−site interactions indeed occur on solid support, with excellent yields and high specificity in certain cases.[17,18] These side reactions can plague solid-phase syntheses where the desired products are those formed in the heterogeneous reaction. Site−site interactions can be reduced by using lower-loaded resins (∼0.05−0.2 mmol/g), using highly cross-linked resins (10−20% cross-linking with divinylbenzene [DVB]), altering the resin linker structure,[18b] or using solvents that do not swell the resins maximally. In this study, we have chosen to work with high capacity (1−2 mmol/g), lightly cross-linked (1% DVB), 500−600 μm polystyrene (PS) beads, which yield ca. 100 nmol of compound per bead upon cleavage.[19] These beads and the linker shown in Scheme 1

Scheme 1



are key elements of a "one bead-one stock solution" technology platform underway at the Harvard ICCB.[20] At the outset, we believed this high-capacity resin could be ideally suited for probing potential site−site interactions.

To test whether intra-site cross-metathesis was feasible on our resin, we selected a template molecule for initial study containing three functionalities: (1) a primary alcohol for facile attachment to beads as a silyl ether, (2) an unhindered terminal olefin to probe cross-metathesis activity, and (3) a masked amine for potential further elaboration. We found that FMOC-*N*-L-serine ω-alkenyl esters (**1a**−**d**) adequately fulfilled these design requirements (Scheme 1). This substrate choice allowed us to vary the length of the olefin tether easily, to investigate a potential length dependence on productive intra-site cross-metathesis. Furthermore, the diagnostic UV chromophore of the FMOC protecting group allowed for quantitative determination of the resin loading.[21]

Synthesis of serine alkenyl esters (**1a**−**d**) with varying olefinic tether lengths (4, 5, 6, and 10 carbons) was straightforward,[21] and each was subsequently loaded onto the PS resin by displacement of a silyl triflate element on

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the linker (Scheme 1).[22] Treatment of the four functionalized resins (**2a**−**d**) with 10 mol % Grubbs' ruthenium benzylidene catalyst **3a**[8] in CH_2Cl_2 (40 °C, 24 h, Ar, with an additional 10 mol % of **3a** added after 12 h) followed by rigorous washing yielded dark brown resins (**4a**−**d**). Cleavage of the silyl ether linker with 5% HF/pyridine in THF, followed by quenching with methoxytrimethylsilane (TMSOMe), yielded pale brown oils.[19] Initial analyses of these oils by reverse-phase HPLC and comparison with the authentic monomeric (**1a**−**d**) and dimeric samples (**5a**,**c**)[23] showed nearly quantitative conversion to new species with retention times identical to that of the respective dimers. ^{1}H NMR and HRMS further confirmed that the predominant products were the dimeric compounds **5a**−**d**.[24]

The *E*/*Z* ratio of the homodimeric products **5a**−**d** and **7a** generated on resin was approximately identical to that of the dimers made in solution (*E*/*Z* ≈ 3:1).[25] Furthermore, the length of the alkenyl tether appeared to have little effect on the efficiency of the intra-site reaction, suggesting that, at this fairly high resin loading, each of the resin-bound monomers was in close proximity to another when the beads were swollen in CH_2Cl_2. Finally, dual FMOC-deprotection of **4a**, carbodiimide coupling with excess benzoic acid, and cleavage generated diamide **7a** quantitatively (Scheme 1), demonstrating that the resin-bound dimers (**4a**−**d**) were amenable to further chemical elaboration.

The results above revealed that intra-site cross-metathesis reactions were indeed possible on our high-capacity resin. However, using a relatively high catalyst loading (20 mol %) had some significant drawbacks. First, the resin became contaminated with dark brown ruthenium catalyst (**3a**) decomposition products[26] that were purged from the resin during the HF cleavage process, thus contaminating the released products. Second, at high catalyst loading, a small percentage of monomer-styrene cross-metathesis product (**6a**−**d**) was always observed, stemming either from the first metathesis catalytic cycle with benzylidene **3a** or from later cross-metathesis of **2a**−**d** with free styrene (Scheme 1).[8,27] Reducing the catalyst load dramatically decreased both of these effects. First, reduction of the catalyst level to 5 mol % appeared to give the highest yield of homodimer **5a** with concurrent reduction in the yield of the styrene adduct **6a** (Figure 2). Second, with the use of less catalyst (**3a**), the beads were no longer markedly discolored after metathesis,

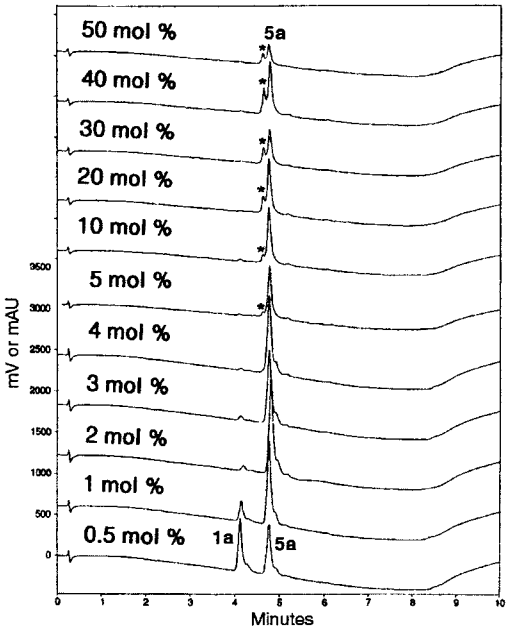

Figure 2. Reverse-phase HPLC traces for crude intra-site cross-metathesis reactions of resin-bound monomer **1a** using various catalyst loadings of benzylidene **3a**. The asterisk marks the peak corresponding to styrene adduct **6a**.

indicating a lesser degree of metal contamination. Metal contamination could be reduced further by treating the resin with $P(CH_2OH)_3$ in CH_2Cl_2/H_2O in accordance with the solution-phase purification procedure recently described by Maynard and Grubbs.[28]

Temperature, solvent, and reaction time optimization studies demonstrated that the optimal conditions for the intra-site cross-metathesis were 5 mol % **3a** (2 × 2.5 mol %) in CH_2Cl_2 (0.03 M in bound substrate) at reflux for 18 h (data not shown). Performing the reactions under a positive pressure of Ar was essential to push the reactions to completion, by removing the metathesis byproduct, ethylene. Notably, after intra-site metathesis, the resin was observed to be more brittle and exhibited reduced swelling capability in organic solvents. We ascribe this change to the dramatic increase in resin cross-linking after the intra-site reaction, and it suggests conducting this transformation at a late stage in library synthesis.

With an optimized intra-site olefin cross-metathesis reaction in hand, we sought to apply this methodology to the split-pool synthesis of a structurally diverse, homodimer library on solid support (Scheme 2). This library is based on three key diversity-generating[29] steps: (1) coupling of unique hydroxybenzaldehyes to the silyl linker, (2) Grignard addition with a reagent having terminal olefin functionality, and (3) acylation or carbamate formation at the newly formed secondary alcohol. Monomeric molecules can be isolated at this juncture by cleavage of a portion of the pooled resin. The remaining resin can be subjected *simultaneously* to intra-site cross-metathesis by treatment with either benzylidene **3a** or new olefin metathesis catalyst **3b**,[30] to generate all of

(22) Cleavage of the FMOC group from aliquots of resin-bound alcohols (**2a**−**d**) with 20% piperidine/DMF and quantitation of the released fulvene product by UV spectroscopy revealed reproducible loadings of 0.7−0.9 mmol/g resin.

(23) Authentic samples of dimers **5a** and **5c** were prepared using the solution-phase cross-metathesis protocol reported in ref 9.

(24) All new compounds exhibited satisfactory spectroscopic data.

(25) *E*/*Z* ratios were determined by integration of ^{1}H NMR spectra; the predominant olefin isomer was assigned as *trans*. See ref 9.

(26) These contaminated polymer beads were not active as immobilized metathesis catalysts; see: Ahmed, M.; Barrett, A. G. M.; Braddock, D. C.; Cramp, S. M.; Procopiou, P. *Tetrahedron Lett.* **1999**, *40*, 8657−8662.

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Scheme 2

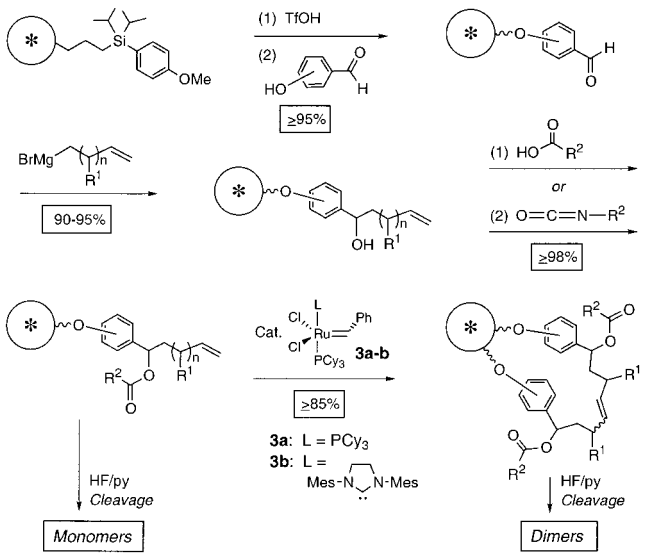
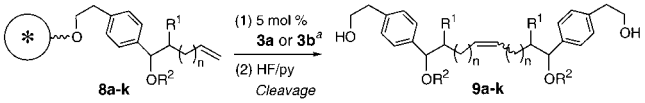


Table 1. Initial Library Screening Reactions



entry	substrate	catalyst[a]	yield %[b]	*E*/*Z*[c]
1: **8a**	$n = 1$, $R^1 = H$, $R^2 = H$	**3a**	**9a**: 32	5:1
2: **8b**	$n = 1$, $R^1 = H$, $R^2 = TBS$	**3a**	**9b**: 96[d]	2:1
3: **8c**	$n = 2$, $R^1 = H$, $R^2 = H$	**3a**	**9c**: 90	5:1
4: **8d**	$n = 2$, $R^1 = H$, $R^2 = TBS$	**3a**	**9d**: 97[d]	4:1
5: **8e**	$n = 1$, $R^1 = H$, $R^2 = OAc$	**3a**	**9e**: 86	3:1
6: **8f**	$n = 2$, $R^1 = H$, $R^2 = OAc$	**3a**	**9f**: 92	2:1
7: **8g**	$n = 1$, $R^1 = H$, $R^2 = C(O)Ph$	**3a**	**9g**: 95	3:1
8: **8h**	$n = 2$, $R^1 = H$, $R^2 = C(O)Ph$	**3a**	**9h**: 98	3:1
9: **8i**	$n = 1$, $R^1 = CH_3$,	**3a**	**9i**: <5	
	$R^2 = C(O)Ph$	**3b**	**9i**: 85	9:1
10: **8j**	$n = 1$, $R^1 = H$,	**3a**[e]	**9j**: 95	2:1
	$R^2 = C(O)NHPh$	**3b**[e]	**9j**: 97	1.3:1
11: **8k**	$n = 2$, $R^1 = H$,	**3a**[e]	**9k**: 98	3:1
	$R^2 = C(O)NHPh$	**3b**[e]	**9k**: 95	2:1

[a] Optimized intra-site metathesis conditions (see text). [b] Determined by ^1H NMR integration of the crude product (relative to starting material) after cleavage. [c] See ref 25. [d] Isolated as the tetraol after HF/py cleavage. [e] 36 h reaction time.

the resin-bound homodimers in one reaction flask. Spatially segregated cleavage from individual beads yields the dimeric molecules (as *E*/*Z* mixtures).[31]

Preliminary results of our building block screening are presented in Table 1. Resin-bound monomers **8a**−**k** were prepared via loading of 4-(2-hydroxyethyl)-benzaldehyde on to our PS resin,[19,20] reaction with various terminal olefin-derived Grignard reagents,[32] and either silyl ether formation, simple acylation, or reaction with phenyl-isocyanate.[33] Protection of the secondary alcohol functionality was observed to promote successful intra-site metathesis with either benzylidene catalyst. The heightened activity[30] of new benzylidene **3b** was required for conversion of allylic-substituted monomer **8i** to dimer **9i**; however, benzylidene **3a** was sufficiently active to generate the other dimer products in good to excellent yields. Finally, as both the Grignard and intra-site metathesis reactions are not stereoselective, solid-phase Brown allylation[34] and olefin hydrogenation[35] chemistry, respectively, are being explored.

We have outlined a general strategy for the synthesis of symmetrical molecules using intra-site reactions on solid support. This methodology could be useful not only for the synthesis of diverse small molecule dimer libraries but also for the on-resin generation of (1) C_2-symmetric metal ligands for asymmetric catalysis and (2) novel, symmetrical peptide architectures. Ongoing work is directed at investigating other intra-site organic transformations on solid support and screening the homodimer library for activity in cell-based, cytoblot assays.[36]

Acknowledgment. We thank the National Institute of General Medical Sciences (GM-52067) for support of this research. The Harvard ICCB is supported by Merck & Co., Merck KGaA, and the National Cancer Institute. H.E.B. is supported by a postdoctoral fellowship from the Jane Coffin Childs Memorial Fund for Medical Research (sponsored by Merck & Co.). S.L.S. is an Investigator at the Howard Hughes Medical Institute.

Supporting Information Available: Representative procedures for intra-site cross-metathesis and library synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(31) All of the library building blocks have been chosen so that the resulting dimeric compounds will have molecular weights in the range of 500−800 g/mol, which could improve their chances of cell permeability. The projected library size is ca. 10 000 members.

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