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SYNTHESIS OF AFFINITY NANOPARTICLES COUPLED TO FR901464 DERIVATIVES

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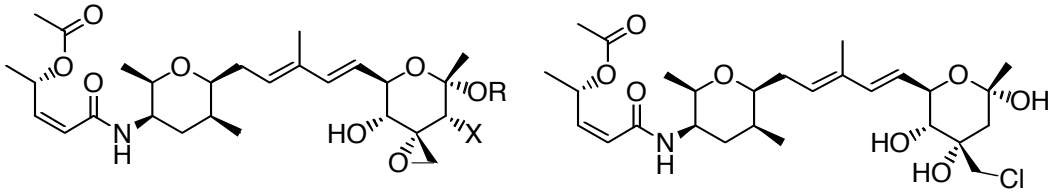
Abstract – The synthesis of two latex nano-particles coupled to FR901464 derivatives is described. Two FR901464 derivatives attached with an amino-alkyl group at a different position were prepared from natural occurring FR 901464. Biological activities of the two ligands were significantly different. The acylation of two amino ligands with the activated esters on latex particles smoothly proceeded to provide the corresponding latex nano-particles coupled to FR 901464 at a different position.

Chemical genomics is an effective methodology for the identifying drug targets using biochemical probes.[1] Biologically active natural products have served as effective biochemical probes since the

This paper is dedicated to Dr. Pierre Potier on the occasion of his 70th Birthday.

structures have already been tuned to bind to their target proteins *in vivo* during evolution.[2] There have been many reports on identification of target proteins of biologically active natural products using solid-supported or biotin-conjugated natural products. However, design and synthesis of the biochemical probes without loss of the biological activity, are often problematic particularly, when the supporting molecule contains several unstable functional groups.

FR 901464 (**1**) and two related compounds (**2**) and (**3**) have been isolated from the culture broth of bacterium *Psudomona* sp. No. 2663 and are composed of two highly functionalized tetrahydropyran rings linked by a diene chain, and an amide side-chain (Figure 1).[3] These compounds were shown to have effects on G_1 and G_2/M cell cycle arrest and to induce DNA fragmentation as well as cell shrinkage during the process of causing cell death. However, the epoxide could be easily opened in the presence of nucleophiles under acidic conditions. The hemiketal moiety is sensitive to basic conditions, undergoing tautmerization to the keto alcohol followed by opining of the epoxide *via* β-elimination. Modification of FR 901464 (**1**) to methyl ether (**4**) is known to improve its stability under basic conditions without the loss of the biological activity. Two research groups have already reported the total synthesis of FR 901464 (**1**). [4,5] However, the structure-activity relationships and the mechanism of the biological action are not still clear. Herein we describe the design and synthesis of a set of solid-supported FR 901464 derivatives for identifying its receptor proteins.



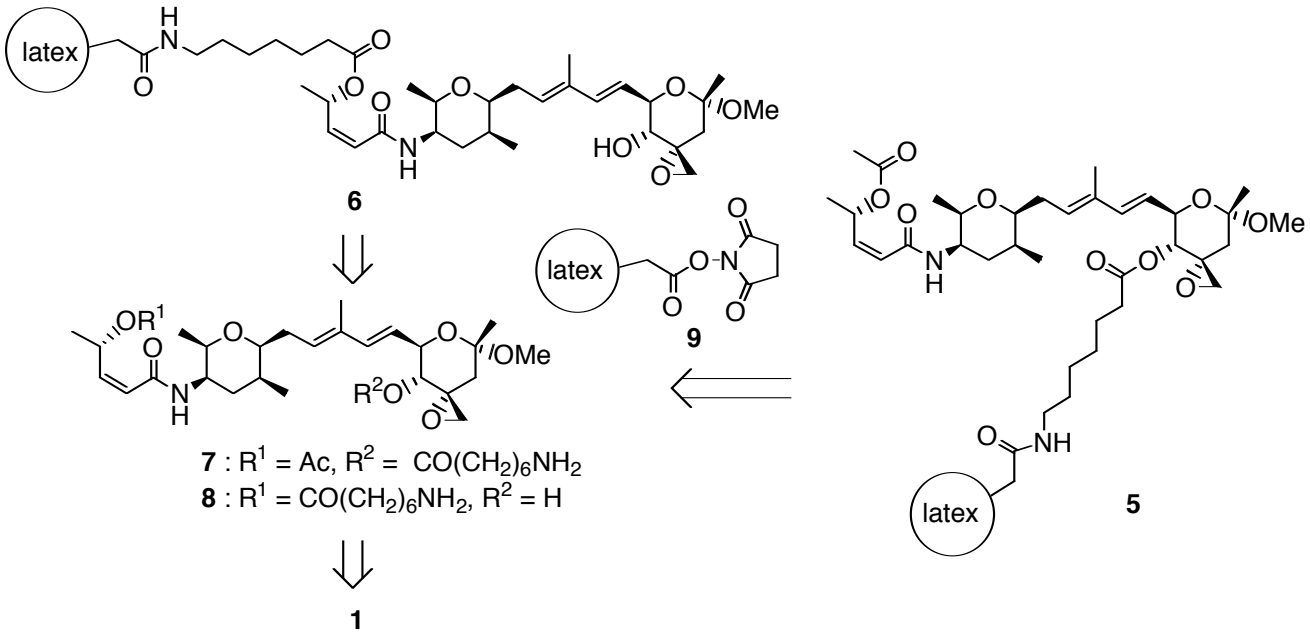
FR 901464 (**1**) : R = H, X = H
2 : R = H, X = OH
4 : R = Me, X = H

3

Figure 1. Structure of FR 901464 (**1**) and two related compounds (**2**) and (**3**)

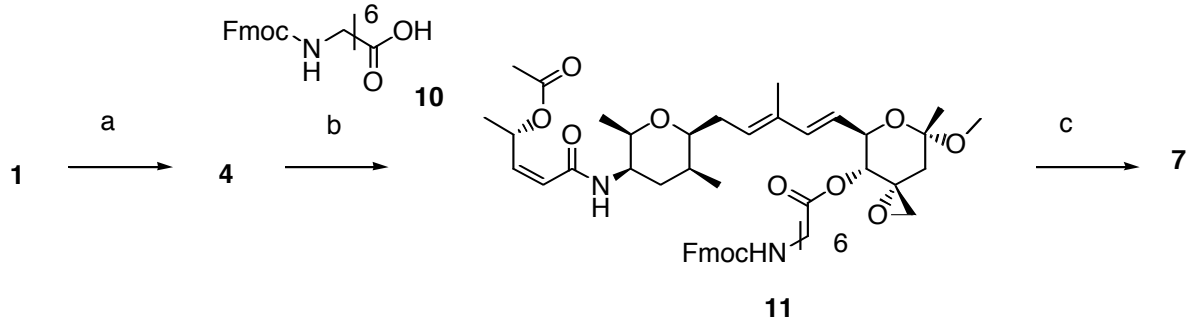
The design and synthesis of the solid-supported FR901464 derivatives is shown in Scheme 1. Two FR901464 methyl ethers (**5**) and (**6**) supported on affinity beads at a different position, are planned to synthesize for its receptor identification. Latex nano-particles composed of stylene-glycidyl methacrylate copolymers for affinity chromatography were selected as the solid-support.[6,7] The poly-glycidyl surface shows relatively little non-specific adsorption of proteins, and can be reacted with amino groups to a load ligands. Furthermore, the large total surface area is especially effective for purifying receptors from a small amount of cell lysate. Recently, we have reported that the synthesis of unique natural product, FR225659 supported on latex nano-particles and application for identification of its receptors.[8] Preparation of the solid-supported FR901464 derivatives (**5**) and (**6**) could easily be achieved by chemoselective acylation of the corresponding amines (**7**) and (**8**) with activated esters on latex nano-

particles. The affinity particles attached with the biologically active ligand could only allow for concentration of the receptor candidates related to the biological activity.



Scheme 1. Strategy for the synthesis of two affinity latex nano-particles coupled to FR901464 derivatives

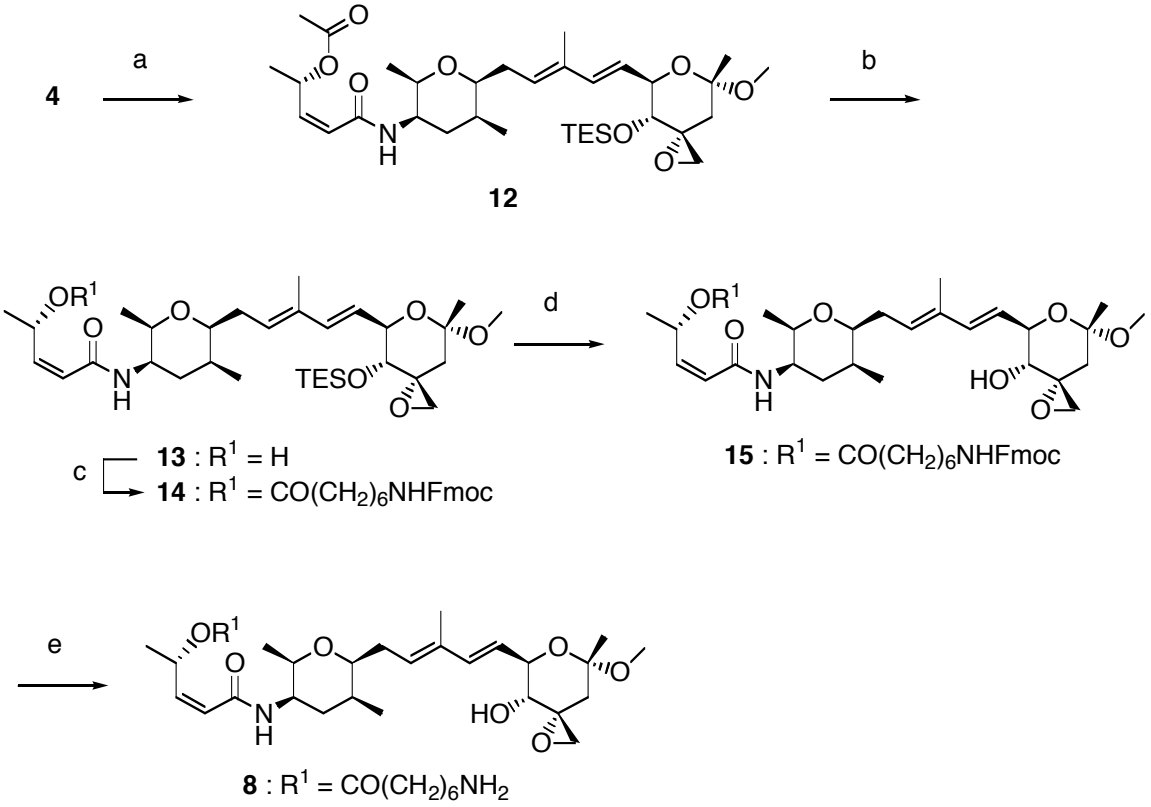
Preparation of amine (**7**) is shown in Scheme 2. Conversion of lactol (**1**) to methyl ether (**4**) in the presence of Amberlyst 15™ in methanol at 0 °C for 3 h provided **4** in 85% yield. Acylation of the hydroxyl group in **4** with the Fmoc protected amino acid (**10**) afforded ester (**11**) in 84% yield. Deprotection of the Fmoc group was accomplished by the treatment of **11** with piperidine to afford amine (**7**) in 72% yield.[9]



Scheme 2. Reagents and conditions: a) Amberlyst 15™, MeOH, 0 °C to rt, 3 h, 85%; b) DCC, DMAP, DCM, rt, 7 h, 84%; c) 20% piperidine, THF, rt, 1 h, 72%;

Preparation of **8** is outlined as shown in Scheme 3. Protection of the hydroxyl group of methyl ether (**4**) with triethylsilyl chloride in the presence of imidazole in DMF at 0 °C for 3 h provided silyl ether (**12**) in 77% yield. Removal of the acetyl group without opening of the epoxide and isomerization of the double bond was successfully accomplished by treatment of **12** with K_2CO_3 in MeOH at 0 °C to room temperature for 3 h to provide alcohol (**13**) in 83% yield. Acylation of alcohol (**13**) with the amino acid

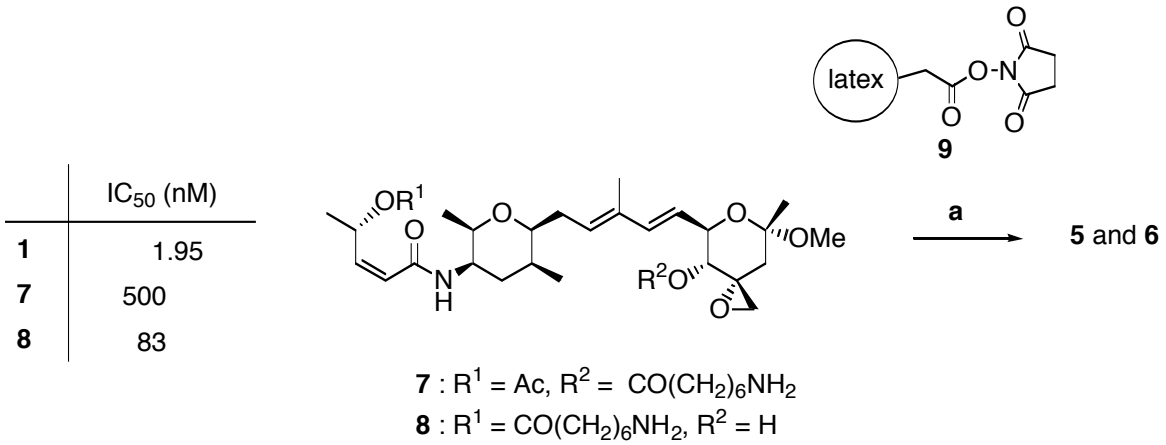
(**10**) using DCC/DMAP in CH_2Cl_2 afforded the Fmoc derivative (**14**) in 89% yield. Deprotection of the triethylsilyl group in **14** with TBAF provided alcohol (**15**) in 65% yield, followed by removal of the Fmoc group with piperidine to provide the amino derivative (**8**) in 60% yield. [10]



Scheme 3. Reagents and conditions: a) TESCl, imidazole, DMF, 0 °C, 3 h, 77%; b) K_2CO_3, MeOH, 0 °C to rt, 3 h, 83%; c) **10**, DCC, DMAP, DCM, 0 °C to rt, 7 h, 89%; d) TBAF, THF, 0 °C to rt, 4 h, 65%; e) 20% peperidine, THF, rt, 2 h, 60%.

The cytotoxicity of amines (**7**) and (**8**) in EL-4 cells was tested (Scheme 4). The amine (**8**) showed the stronger activity (IC_{50} = 83 nM) than amine (**7**) (IC_{50} = 500 nM). The biological activity of amine (**8**) attached on the side-chain was lower than that of FR901464 (**1**) (IC_{50} = 1.98 nM), and but would be an acceptable level of biological activity to make it of potential value for use in affinity supported receptor identification. Next, the synthesis of he affinity particles (**5**) and (**6**) coupled with different amounts of FR901464 from the amino ligands (**7**) and (**8**) was examined. Treatment of the activated esters (**9**) on the latex particles with two DMF solutions of amines (**7**) and (**8**) (2.0 and 20 mM) at room temperature for 24 h, followed by exposure of the resulting particles with 2-hydroxylethylamine to provided affinity particles (**5**) and (**6**), respectively. [11] The advantage of the use of 2-hydroxylethylamine is to reduce non-specific protein adhesion of the particles by transformation of the remaining activated esters to the corresponding amide. Loading amount of the ligands on the particles (**5**) and (**6**) was estimated by quantitative analysis of released hydroxyl succinimide in the coupling reaction based on UV absorption at 214 nm to be 26 and 56 nmol/mg for **5** and 34 and 53 nmol/mg for **6**, respectively. 2-Hydroxylethylamine would not undergo

nucleophilic addition to epoxide (**5**) and (**6**) under these reaction conditions because epoxide (**2**) was stable to the same conditions.



Scheme 4. Reagents and conditions: a) **7** or **8**, DMF, rt, 1 h then $HOCH_2CH_2NH_2$, DMF, rt, 1 h.

In conclusion, we describe the synthesis of the latex nano-particles (**5**) and (**6**) coupled with the different FR 901464 derivatives (**7**) and (**8**). The amino ligands (**8**) attached with the amine on the side-chain showed stronger biological activity than **7**, and however, exhibited weaker comparison with FR 901464 (**1**). The coupling reaction of the two amino ligands with activated esters on the latex particles smoothly proceeded to provide the corresponding affinity particles (**5**) and (**6**) with different loading amount. The latex particles with different loading levels of the ligands should be effective tools to identify specific binding proteins to FR901464. Receptor identification of FR901464 using the latex nano-particles (**5**) and (**6**) is in progress.

ACKNOWLEDGEMENTS

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9. Spectra of **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (d, 3H, *J* = 7.3 Hz), 1.14 (d, 3H, *J* = 6.3 Hz), 1.35-1.26 (m, 4H), 1.38-1.37 (m, 3H), 1.39 (s, 3H), 1.52-1.47 (m, 2H), 1.70-1.67 (m, 2H), 1.72 (d, 1H, *J* = 14.2 Hz), 1.72 (s, 3H), 1.95-1.92 (m, 2H), 2.03 (s, 3H), 2.27-2.17 (m, 3H), 2.33 (d, 1H, *J* = 14.6 Hz), 2.41-2.31 (m, 1H), 2.48 (d, 1H, *J* = 4.4 Hz), 2.60 (d, 1H, *J* = 4.4 Hz), 3.18-3.15 (m, 2H), 3.29 (s, 3H), 3.51-3.48 (m, 1H), 3.66-3.64 (m, 1H), 3.94-3.91 (m, 1H), 4.23-4.20 (m, 1H), 4.41-4.37 (m, 3H), 4.83-4.81 (m, 1H), 5.51-5.46 (m, 2H), 5.70 (d, 1H, *J* = 11.7 Hz), 5.87 (dd, 1H, *J* = 11.7, 7.8 Hz), 5.99 (d, 1H, *J* = 8.8 Hz), 6.28-6.24 (m, 1H), 6.31 (d, 1H, J = 16.1 Hz), 7.31 (dd, 2H, *J* = 7.3, 7.8 Hz), 7.40 (dd, 2H, J = 7.3, 7.8Hz), 7.59 (d, 2H, J = 7.8 Hz), 7.76 (d, 2H, J = 7.3 Hz); MS (ESI-TOF) 871 $[M+H]^+$.
10. Spectra of **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (d, 3H, *J* = 7.0 Hz), 1.11 (d, 3H, *J* = 6.3 Hz), 1.33-1.22 (m, 4H), 1.34-1.33 (m, 3H), 1.36 (s, 3H), 1.60-1.40 (m, 5H), 1.68 (s, 3H), 1.86-1.75 (m, 2H), 2.01 (s, 3H), 2.39-2.15 (m, 5H), 2.45 (d, 1H, *J* = 4.3 Hz), 2.57 (d, 1H, *J* = 4.3 Hz), 3.25 (s, 3H), 3.29-3.27 (m, 2H), 3.48-3.46 (m, 1H), 3.63-3.62 (m, 1H), 3.92-3.90 (m, 1H), 4.22-4.20 (m, 1H), 5.47-5.41 (m, 2H), 5.69 (d, 1H, *J* = 11.7 Hz), 5.86 (dd, 1H, *J* = 11.2, 8.3 Hz), 6.0 (d, 1H, *J* = 8.8 Hz), 6.29-6.21 (m, 2H); MS (ESI-TOF) 649 $[M+H]^+$.