Organic & Biomolecular Chemistry

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Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID	OB-ART-11-2018-002900.R1
Article Type:	Paper
Date Submitted by the Author:	19-Dec-2018
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PAPER

Design and Synthesis of N^1,N^8-Diacetylspermidine Analogues Having A Linker with Desired Functional Groups

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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A synthesis of new N^1,N^8-diacetylspermidine (DiAcSpd) analogues having a linker with desired functional groups in the methylene skeleton, which have been designed by theoretical calculation, is described. We have also achieved the preparation of DiAcSpd supported on solid-phase resins, which have the potential to be used for evolution of ligands by exponential enrichment (SELEX).

Introduction

Polyamines, such as putrescine, spermidine, or spermine, are low-molecular-weight and physiologically active compounds in which some of the amino groups are contained in an alkyl chain skeleton (Figure 1A). They play many important roles in cell proliferation and differentiation.[1] It is known that cell proliferation is significantly affected when polyamines are deficient or in excess, therefore, their intracellular concentration is strictly controlled. It was furthermore estimated the polyamine distribution in cells, they interact mainly with RNAs of nucleic acids and have been shown to be greatly involved in protein synthesis.[2] In particular, spermidine and its derivatives have been well studied because they are involved in human health and diseases.[3] It is generally agreed upon that N^1,N^8-diacetylspermidine (DiAcSpd), which is a metabolite of spermidine via acetyltransferase, has the potential to be a biomarker for the diagnosis of malignancies.[4] The amount of DiAcSpd is remarkably increased in the urine of cancer patients, whereas it is hardly elevated in the case of a healthy person, which means that the urinary level of DiAcSpd is a useful diagnostic and prognostic indicator for cancer. The development of a system for the easy and rapid detection of DiAcSpd in the urine is therefore required. For example, in 1997, Kawakita *et al*. reported the preparation of antibodies with high specificity to DiAcSpd.[5]

In recent years, high-affinity oligonucleotides called 'aptamers' have drawn attention as biomolecules that highly recognize the three-dimensional structure of the target molecule.[6] They can be obtained from randomized RNA libraries (ca. ~10^{14} oligonucleotides) by *in vitro* selection, which is termed 'Systematic Evolution of Ligands by EXponential enrichment '(SE LEX).[7] Aptamers also have properties that are not present in antibodies: (1) they are easy to chemically modify and (2) Their molecular weight are smaller and they can be synthesized in large amounts. Aptamers are therefore suitable as diagnostic materials. In general, obtaining aptamers that target low-molecular-weight compounds is difficult and seldom reported. In our previous work, we succeeded in obtaining two RNA aptamers targeted for spermine by SELEX.[8] We also looked at obtaining DiAcSpd-specific aptamers in the same way as we did with spermine, but unfortunately we were unsuccessful (Figure 1B).[9] This is probably because the aptamers could not recognize the three-dimensional structure and functional groups of DiAcSpd due to DiAcSpd being immobilized on the solid-phase resin using one nitrogen atom of the substrate as a foothold. In SELEX, it is common to use analogues that target molecules immobilized on solid-phase resin as a support, and many aptamers are obtained by this technique. Based on the above study, we set out to design the analogues whose conformation and functional groups would be maintained as in the DiAcSpd.

We then hypothesized that DiAcSpd analogues having a linker in the methylene skeleton could keep the same or similar three-dimensional structure and functional groups as DiAcSpd (Figure 1C). To the best of our knowledge, this type of spermidine analogue has been overlooked and remain unreported, while many other spermidine analogues, including orthogonally protected or with side chains grown from the internal nitrogen atom of the substrate, have been reported.[10] Not limited to polyamines, many bioactive compounds, such as histamine, serotonin, or adrenaline, use methylene skeletons to connect functional groups. It is important, therefore, to develop a synthesis of analogues having a linker with desired functional groups in the methylene skeleton in order to obtain the corresponding biomolecules.

In this study, we describe a synthesis to produce new DiAcSpd analogues **1** and **2** which have a linker with desired functional groups, such as hydroxide or amine groups, in the linker

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

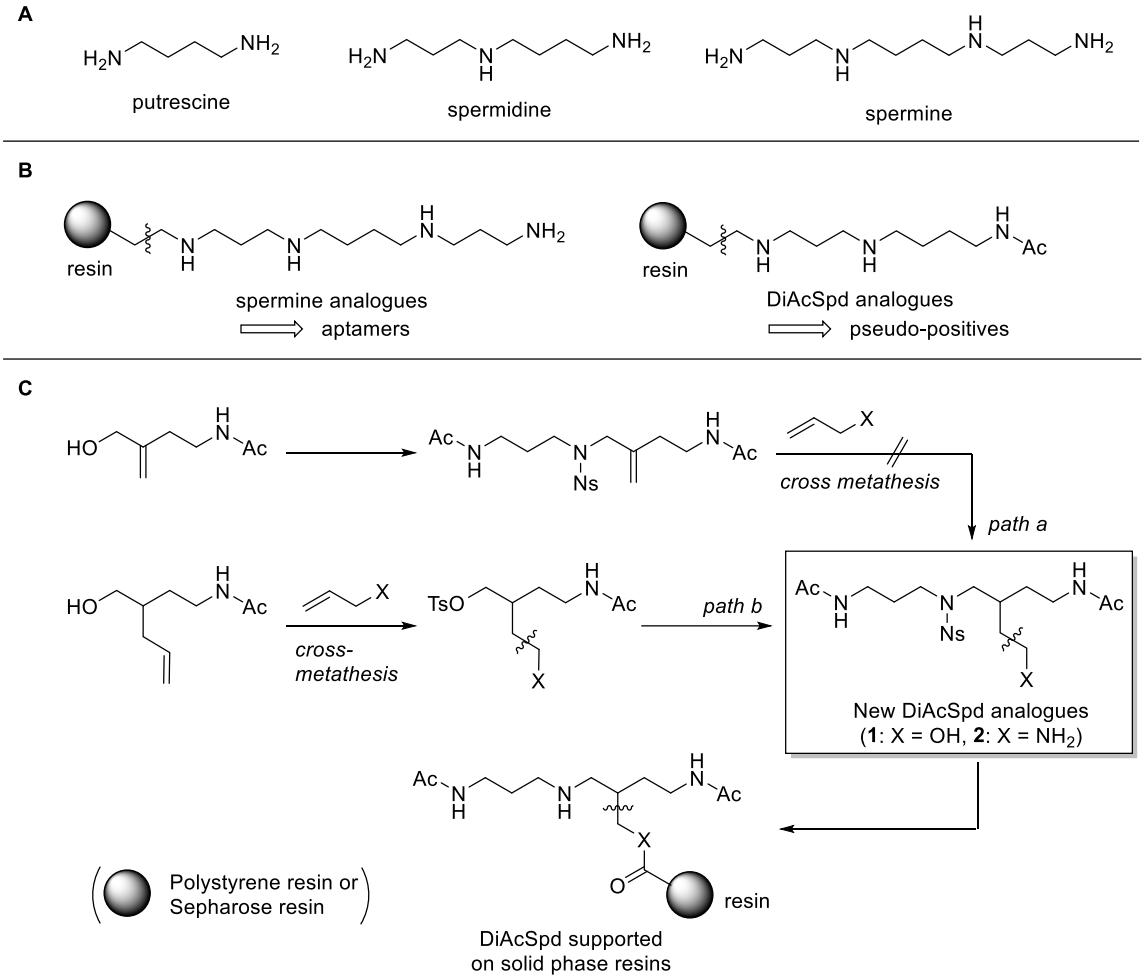


Figure 1 (A) Structures of representative polyamines: putrescine, spermidine, and spermine. (B) Our previous works. (C) This work: synthesis of new DiAcSpd analogues having a linker with desired functional groups in the methylene skeleton and DiAcSpd supported on solid-phase resins.

instead of the resin structure. It was observed that substitution at the α-position of the amino groups, such as the methylene skeleton that have been designed based on conformational studies using theoretical calculations. Furthermore, we synthesized DiAcSpd analogues supported on solid-phase resins that have the potential to be used for SELEX.

Results and discussion

First of all, we investigated the conformational study of DiAcSpd and its analogues having a linker in methylene skeleton in order to understand in which position the linker should be introduced. Our investigation revealed that DiAcSpd should adopt a linear conformation as its stable conformation (Figure 2A). We then performed conformational studies of DiAcSpd analogues having a linker in the methylene skeleton and in order to simplify the calculation, a butyl group was used as the model linker instead of the resin structure. It was observed that substitution at the α-position of the amino groups, such as the C5 and C8 positions, induced the DiAcSpd analogue into a bent conformation which greatly differed from the original (Figure 2B). These results suggest that it would be better to avoid introducing a linker at the α-position of each of the amino groups. We also examined the conformational effects of DiAcSpd analogues having a linker at the β-position of each of the amino groups, such as the C6 and C7 positions (Figure 2C). Substitution at the C7 position resulted to a non-linear conformation, which was caused by the hydrogen bond formation between acetoamide and internal amine. On the other hand, substitution at the C6 position led to a roughly linear conformation which was similar to the stable conformation of DiAcSpd. We therefore examined the synthesis of DiAcSpd analogues having a linker at the C6 position since we presumed that this design of the compound would keep a similar three-dimensional structure and functional groups as in DiAcSpd. We then developed two synthetic routes for synthesizing for synthesizing DiAcSpd analogues having a linker with desired functional groups at the C6 position (Figure 1C).

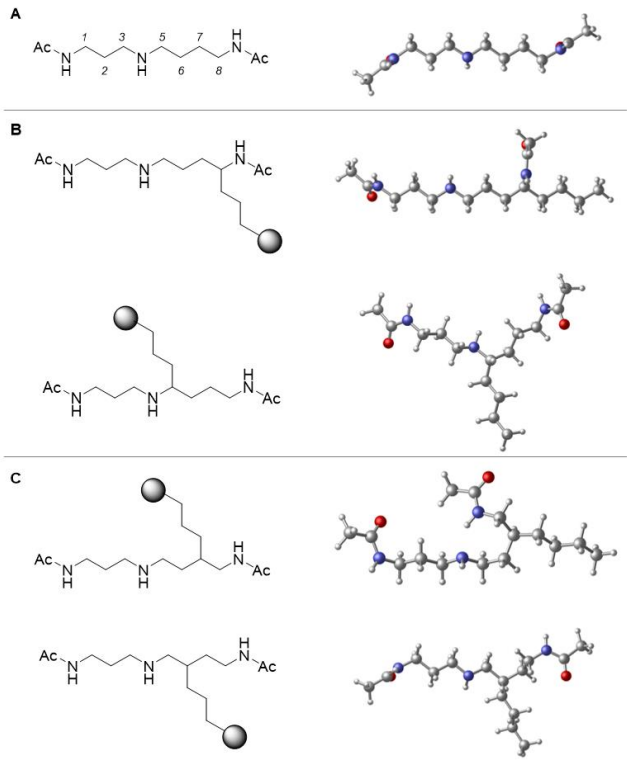
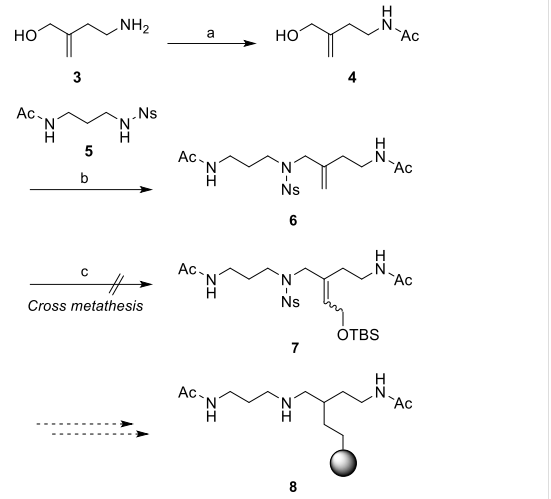
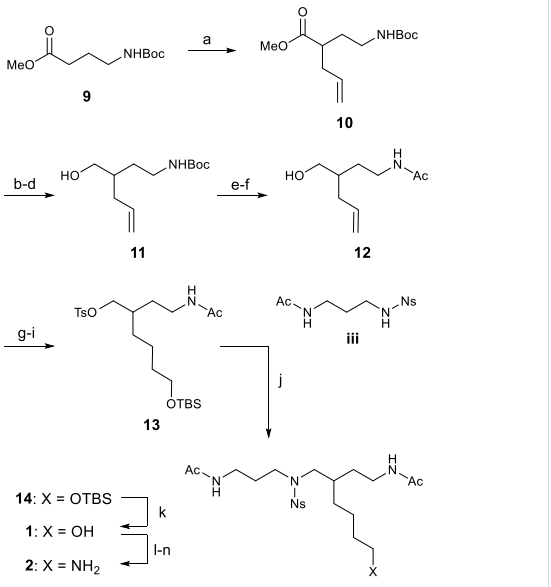


Figure 2 Conformational modeling performed with MCMM (OPLS-2005) using MacroModel 10.8 and DFT (B3LYP/6-31G(d) using Gaussian 09: (A) DiAcSpd; (B) DiAcSpd with a linker at the α-position of each of the amino groups; (C) DiAcSpd with a linker at the β-position of each of the amino groups. CYLview was used for the three-dimensional optimized structures: gray, C; white, H; red, O; blue, N.

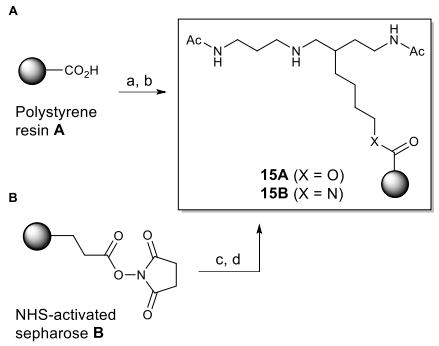


Scheme 1 Synthesis route for DiAcSpd analogue **8**.
Reagents and conditions: (a) Ac_2O, DCM, 0 °C to r.t.; (b) DEAD, PPh_3, toluene, 80 °C; (c) Ru cat., (allyloxy)(tert-butyl)dimethylsilane, CH_2Cl_2, reflux.; DEAD = diethyl azodicarboxylate.



Scheme 2 Synthesis of DiAcSpd analogues **1** and **2**.
Reagents and conditions: (a) $LiN(SiMe_3)_2$, allylbromide, THF, -78 °C; (b) LiOH, THF; (c) $ClCO_2Et$, Et_3N, THF, -10 °C; (d) $NaBH_4$, THF/H_2O, 0 °C to r.t.; (e) 4 M HCl/1,4-dioxane, Et_2O; (f) Ac_2O, Et_3N, CH_2Cl_2, 0 °C to r.t.; (g) Grubbs 1st, (allyloxy)(*tert*-butyl)dimethylsilane, CH_2Cl_2, reflux; (h) PtO_2, EtOAc, H_2 (5 bar); (i) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to r.t.; (j) **5**, Cs_2CO_3, DMF, 80 °C; (k) *n*-Bu_4NF, THF, 0 °C to r.t.; (l) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to r.t.; (m) NaN_3, DMF, 80 °C; (n) PPh_3, THF/H_2O.

First, path a, was examined. Its strategy consisted of constructing the spermidine skeleton first and then extending the side chain moiety by metathesis reaction. We predicted that DiAcSpd analogue **7** which has a linker could be prepared from commercially available 4-amino-2-methylenebutan-1-ol (**3**) in just two steps (Scheme 1). Treatment of **3** with acetic anhydride in dichloromethane affords the acetamide **4**, which was subjected to reaction with o-nitrobenzenesulfonyl (Ns) amide **5** that contained an acetyl group to construct the DiAcSpd skeleton with an exo-methylene substituent in the methylene skeleton (**6**). Next, we conducted the cross-metathesis of DiAcSpd analogue **6** with allylic alcohol derivatives using commercially available ruthenium catalysts, such as Grubbs 1st, Grubbs 2nd, Hoveyda-Grubbs 1st, and Hoveyda-Grubbs 2nd. However, the cross-metathesis product (**7**) was not observed at all. We attributed this result to the competition between the productive cross-metathesis and rapid self- cross-metathesis of allylic alcohol derivatives. We speculated that the steric bulkiness imposed by the Ns amide group at the β-position hindered the reaction of substrate (**6**) and disfavoured formation of the metallocyclobutane intermediate, resulting to the lack of productive cross-metathesis to afford **7**. As is often the case with cross-metathesis, the reactivity of sterically demanding alkene is a lot poorer than terminal olefins.[10] Of course, several syntheses of trisubstituted olefins by cross-



Scheme 3 Loading of DiAcSpd analogues on solid-phase resin. (A) DiAcSpd analogue **1** on polystyrene-COOH resin A; (B) DiAcSpd analogue **2** on NHS-activated sepharose B.
Reagent and conditions: (a) **1**, EDC · HCl, DMAP, CH_2Cl_2; (b) DBU, 2-mercaptoethanol, DMF; (c) 10 mM of **2** in Milli-Q water; (d) Cs_2CO_3, 2-mercaptoethanol.

metathesis using sterically demanding alkenes such as 1,1-disubstitued alkenes as a substrate have been reported.[11] However, this type of reaction remains challenging and is limited to simple substrates without functional groups. Therefore, we decided to carry out the cross-metathesis in the initial stage of synthesis In addition, we decided to change the exo-methylene moiety of **4** to an allylic one (**12**) in order to improve the reactivity of cross-metathesis.

Next, we examined the alternative synthesis route (Figure 1C, path b), In this pathway, we extended the side chain moiety by metathesis reaction first and then the spermidine skeleton was constructed. We supposed that this pathway could solve the low reactivity of the metathesis reaction which was the weakness of path a. As shown in Scheme 2, DiAcSpd analogues **1** and **2**, which satisfied the required molecular design based on prior conformational studies, were synthesized from commercially available methyl 4-((*tert*-butoxycarbonyl)amino)butanoate (**9**). We started the synthesis by reacting **9** with allylbromide to give adduct **10**. Reduction of **10** via mixed acid anhydride then gave alcohol **11** in 98% yield. The *tert*-butoxycarbonyl (Boc) group in **11** was deprotected with HCl and subjected to acetylation to afford **12** in 81% yield over two steps. Cross-metathesis of **12** with (allyloxy)(*tert*-butyl)dimethylsilane proceeded smoothly with Grubbs 1st as the catalyst to give the metathesis product.

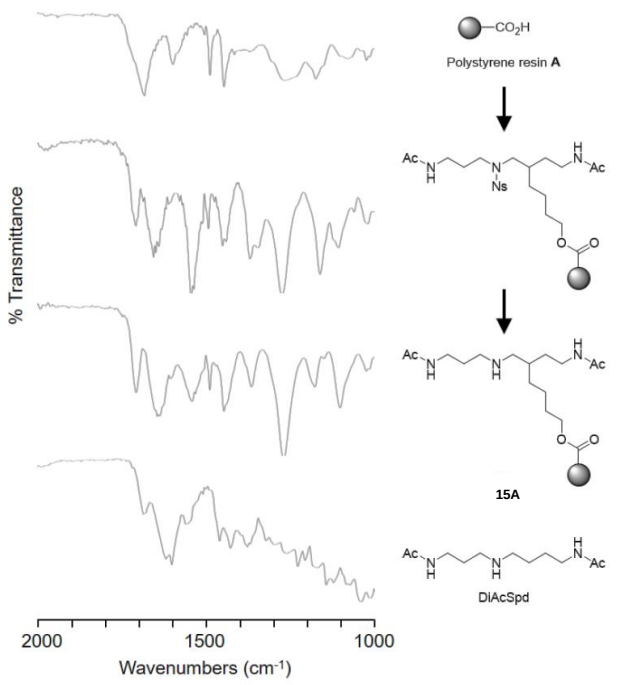


Figure 3 Monitoring of the immobilization of DiAcSpd on polystyrene-COOH resin by ATR-IR.

Subsequently, catalytic reduction of the metathesis product proceeded with PtO_2 under a hydrogen atmosphere. This was then followed by tosylation of the alcohol to give **13** in 57% yield over three steps. Upon treatment of **13** with the Ns amide **iii** and Cs_2CO_3, the desired coupling reaction proceeded to afford DiAcSpd skeleton **14** in 83% yield. Deprotection of (*tert*-butyl)dimethylsilyl (TBS) was carried out by treatment with tetra-*n*-butylammonium fluoride in tetrahydrofuran (THF) to give the DiAcSpd with hydroxy linker **1**. Upon further treatment of **1** with *p*-toluenesulfonyl (Ts) chloride, triethylamine, and4-dimethylaminopyridine (DMAP), followed by reaction with NaN_3 in dimethylformamide (DMF), afforded the azide product. Subsequently, the azide product was treated with triphenylphosphine in THF/H_2O (Staudinger reaction) to afford DiAcSpd with amine linker **2** in 64% yield over three steps.

We then turned our attention to the loading of DiAcSpd analogues on a solid-phase resin. At first, polystyrene resin that has a carboxylic acid such as commercially available polystyrene- COOH resin A (Watanabe Chemical Industries, Ltd) was used as a support (Scheme 3A). Based on literature method, DiAcSpd analogue with hydroxyl linker **1** was attached to polystyrene resins by treatment with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC · HCl) as a condensation reagent and DMAP.[13] The solid-phase reaction was monitored by attenuated total reflectance infrared spectroscopy (ATR-IR) (Figure 3).[14] The product was confirmed based on the appearance of the absorption peak of the Ns group (*ca.* 1550 cm^{-1}). Subsequently, the deprotection of the Ns group

was accomplished by treatment with 2-mercaptoethanol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford the resin-bound DiAcSpd analogue **15A**. The product was then confirmed based on disappearance of the absorption peak of the Ns group (*ca.* 1550 cm^{-1}), and the Kaiser test using ninhydrin to check the loading of amines.[15] Next, a sepharose resin, NHS-activated sepharose (GE Healthcare Life Sciences), was used as support similar to polystyrene resin (Scheme 3B). In SELEX, the use of different types of resin is effective for subtracting the pseudo-positive aptamers that bind to solid-phase resins. DiAcSpd

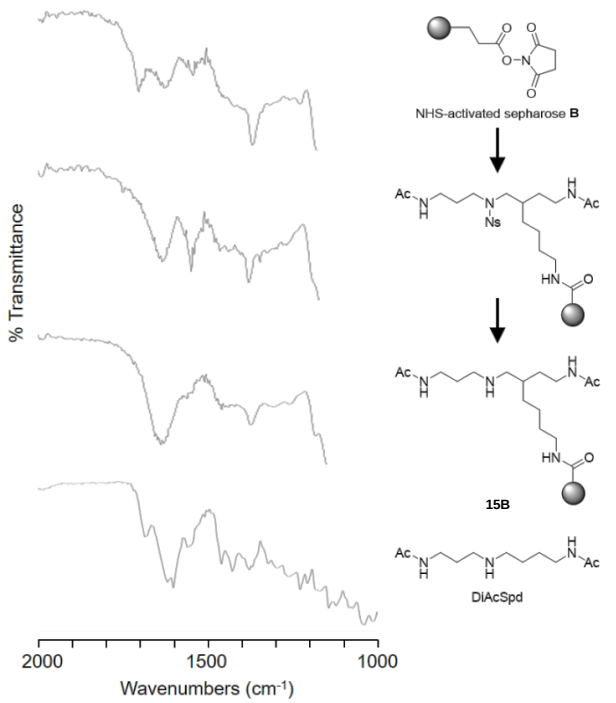


Figure 4 Monitoring of the immobilization of DiAcSpd on NHS-activated sepharose resin by ATR-IR.

analogue having amine linker **2** was immobilized on NHS-activated sepharose by amine coupling through its primary amino group. Subsequently, deprotection of the Ns group was accomplished by treatment with 2-mercaptoethanol and Cs_2CO_3 to afford resin-bound DiAcSpd analogue **15B**. These reactions were also monitored by ATR-IR, and the products were confirmed based on the appearance or disappearance of the absorption peak of the Ns group (Figure 4).

We are currently examining SELEX in detail in order to obtain DiAcSpd-specific aptamers using DiAcSpd analogues **15A** and **15B**. We are also investigating their biochemical and physicochemical properties which will be reported in due course.

Conclusion

In conclusion, we have developed a synthesis to produce new DiAcSpd analogues that have a linker with desired functional groups, such as hydroxide or amine in the methylene skeleton. Furthermore, it was possible to immobilize these analogues on solid-phase resins for potential use in SELEX.

Experimental

General experimental details

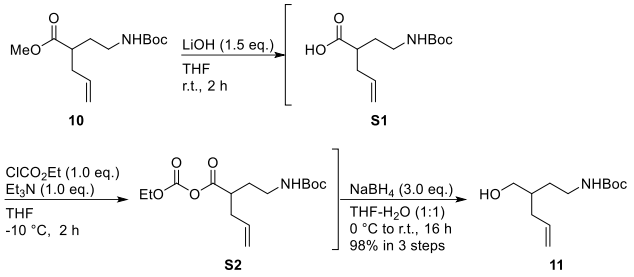
All reagents were purchased from commercial sources and used without further purification, unless otherwise noted. Reactions were performed under a nitrogen atmosphere using purchased anhydrous solvent. All reactions were monitored by thin-layer chromatography using Merck SiO_2 gel 60 F254. The products were purified by column chromatography over SiO_2 60N (40-50 µm, spherical neutral) purchased from Kanto Chemical. ^1H NMR and ^{13}C NMR spectra were recorded at 25 °C on a JEOL JNM-AL300 (at 300 MHz and 75 MHz, respectively), a JEOL JNM-ECS 400 (at 400 MHz and 100 MHz, respectively) or a JEOL JNM-LA 500 (at 500 MHz and 125 MHz, respectively), and the chemical shifts are reported relative to internal TMS (^1H, δ = 0.00 ppm), $CDCl_3$ (^1H, δ = 7.26 ppm, ^{13}C, δ = 77.0 ppm), CD_3OD (^1H, δ = 3.31 ppm, ^{13}C, δ = 49.0 ppm). Data for ^1H NMR spectra are reported as follows: chemical shift (δ ppm) (integration, multiplicity, coupling constant (Hz)). Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. IR spectra were recorded with Shimadzu IRAffinity-1 spectrometers equipped with Specac Quest Single Reflection Attenuated Total Reflection (ATR) Accessory (P/N GS 10800). High-resolution mass spectra (MALDI-TOF) were performed by the Elemental Analysis Section of Graduate School of Pharmaceutical Science in Osaka University. **5**[16] and **9** (commercially available) are known compounds. DiAcSpd was synthesized based on the method of the literature[17].

Experimental procedures

10: To a solution of **9** (835.0 mg, 3.85 mmol) in THF (15.4 mL) was added dropwise potassium bis(trimethylsilyl)amide (ca. 0.5 M in toluene, 20 mL, 10.0 mmol, 2.6 equiv.) at −78 °C. After 0.5 h, the reaction mixture was added dropewise allyl bromide (1.6 mL, 19.2 mmol, 5.0 equiv.), and the mixture was stirred at the same temperature for 6 h. Then, sat. NH_4Cl aq. was added, and the mixture was warmed to room temperature, the organic layer was separated and the aqueous layer was extracted with EtOAc. The organic layers were combined, and the solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 9/1 to 5/1) to give **10** (404.1 mg, 1.57 mmol, 40%) as a colorless oil.

^1H NMR (400 MHz, $CDCl_3$) δ ; 5.71 (1H, ddt, *J* = 17.4, 10.1, 6.9 Hz), 5.08-5.00 (2H, m), 4.57 (1H, brs), 3.67 (3H, s), 3.21-3.05 (2H, m), 2.53-2.46 (1H, m), 2.41-2.34 (1H, m), 2.28-2.21 (1H, m), 1.84-1.75 (1H, m), 1.72-1.64 (2H, m), 1.43 (9H, s); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.7, 155.8, 134.9, 117.1, 19.2, 51.6, 42.8, 38.6, 36.3, 31.7, 28.3; **HRMS** (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{13}H_{23}NO_4Na$ 280.1519, found 280.1511.

11:



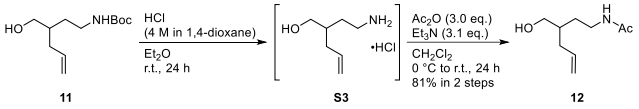
(Step 1): To a solution of **10** (1.04 g, 4.04 mmol, 1.0 equiv.) in THF (13.5 mL) was added 1 M LiOH aq. (6.06 mL, 6.06 mmol, 1.5 equiv.) at room temperature, and the mixture was stirred for 24 h. Then, 1 M HCl aq. and EtOAc was added. The organic layer was separated and the aqueous layer was extracted with EtOAc. The organic layers were combined, and the solution was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the crude carboxylic acid **S1** (0.982 g, 4.04 mmol).

(Step 2): To a solution of **S1** (0.982 g, 4.04 mmol) in THF (13.5 mL) was added ethyl chloroformate (0.39 mL, 4.04 mmol, 1.0 equiv.) and Et_3N (0.42 mL, 4.04 mmol, 1.0 equiv.) at −10 °C, and the mixture was stirred for 2 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure to give the crude acid anhydride **S2** (1.27 g, 4.04 mmol).

(Step 3): To a solution of **S2** (1.27 g, 4.04 mmol) in THF (6.5 mL) and H_2O (6.5 mL) was added $NaBH_4$ (458.5 mg, 12.1 mmol, 3.0 equiv.) in an ice bath, and the mixture was stirred at room temperature for 16 h. Then, the organic layer was separated and the aqueous layer was extracted with EtOAc. The organic layers were combined, and the solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/1) to give **11** (0.907 g, 3.96 mmol, 98% in 3 steps) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (1H, ddt, J = 17.4, 10.1, 6.8 Hz), 5.11-5.02 (2H, m), 3.64-3.53 (2H, m), 3.25-3.12 (2H, m), 2.15-2.04 (2H, m), 1.70-1.50 (3H, m), 1.50 (9H, s); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 156.3, 136.5, 116.6, 79.3, 65.1, 38.6, 37.9, 35.9, 31.4, 28.4; **HRMS** (MALDI-TOF) m/z $[M+Na]^+$ calcd for $C_{12}H_{23}NO_3Na$ 252.1570, found 252.1567.

12:

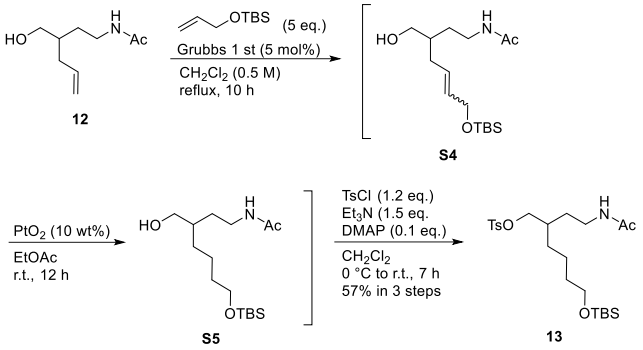


(Step 1): To a solution of **11** (252.0 mg, 1.10 mmol, 1.0 equiv.) in Et_2O (1.8 mL) were added HCl (4 M in 1,4-dioxane, 1.1 mL, 4.4 mmol, 4.0 equiv.) at room temperature, and the mixture was stirred for 24 h. Then, the solution was concentrated under reduced pressure to give the crude amine **S3** (184.0 mg, 1.10 mmol).

(Step 2): To a solution of **S3** (184.0 mg, 1.10 mmol) in CH_2Cl_2 (5.5 mL) was added Ac_2O (0.31 mL, 3.30 mmol, 3.0 equiv.) and Et_3N (0.47 mL, 3.41 mmol, 3.1 equiv.) in an ice bath, and the mixture was stirred for 24 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30/1 to 20/1) to give **12** (152.4 mg, 0.889 mmol, 81% in 2 steps) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (1H, brs), 5.77 (1H, ddt, J = 17.2, 10.3, 6.9 Hz), 5.07-5.02 (2H, m), 3.65 (1H, dd, J = 10.9, 4.6 Hz), 3.65 (1H, dd, J = 10.9, 6.3 Hz), 3.39-3.24 (2H, m), 2.41 (1H, brs), 2.14-2.02 (2H, m), 1.98 (3H, s), 1.71-1.63 (1H, m), 1.59-1.54 (1H, m); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.5, 136.4, 116.7, 65.3, 38.0, 37.7, 36.0, 31.0, 23.2; **HRMS** (MALDI-TOF) m/z $[M]^+$ calcd for $C_9H_{18}N_2O_2$ 172.1332, found 172.1332.

13:



(Step 1): To a solution of **12** (227.0 mg, 1.325 mmol, 1.0 equiv.) in degassed CH_2Cl_2 (2.6 mL) were added (allyloxy)(*tert*-butyl)dimethylsilane (1.14 g, 6.63 mmol, 5.0 equiv.) and Grubbs 1st (54.5 mg, 0.066 mmol, 5 mol%) at room temperature. Then, the reaction mixture was warmed to reflux. After stirring for 10 h, the reaction mixture was cooled to room temperature, and then the mixture was concentrated under reduced pressure. The residue was passed through a short silica gel column ($CHCl_3$/MeOH = 30/1 to 25/1) to give the crude metathesis product **S4** (263.5 mg, 0.835 mmol).

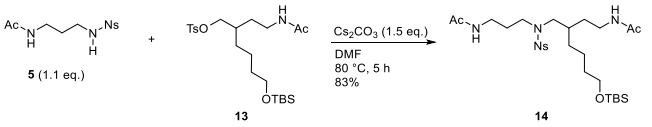
(Step 2): To a solution of **S4** (263.5 mg) in EtOAc (8.4 mL) was added PtO_2 (26.4 mg, 10 wt%) at room temperature under an H_2 atmosphere (balloon), and the mixture was stirred for 12 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure to give the crude **S5** (265.1 mg, 0.835 mmol).

(Step 3): To a solution of **S5** (265.1 mg, 0.835 mmol, 1.0 equiv.) in CH_2Cl_2 (4.2 mL) was added TsCl (190.9 mg, 1.00 mmol, 1.2 equiv.), Et_3N (0.17 mL, 1.25 mmol, 1.5 equiv.) and DMAP (10.2 mg, 0.084 mmol, 0.1 equiv.) in an ice bath, and the mixture was stirred at room temperature for 7 h. Then, water was added, and the organic layer was separated and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined, and the solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30/1 to 25/1) to give **13** (356.2 mg, 0.755 mmol, 57% in 3 steps) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, J = 8.3 Hz), 7.35 (2H, d, J = 8.3 Hz), 5.59 (1H, brs), 4.00-3.86 (2H, m), 3.53 (2H, t, J = 6.4 Hz), 3.26-

3.15 (2H, m), 2.45 (3H, s), 1.95 (3H, s), 1.71-1.66 (1H, m), 1.54-1.49 (2H, m), 1.45-1.38 (2H, m), 1.32-1.19 (4H, m), 0.97 (9H, s), 0.02 (6H, s); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.1, 144.9, 132.7, 129.9, 127.8, 62.8, 37.1, 35.7, 32.7, 30.7, 30.4, 25.9, 23.3, 22.7, 21.6, 18.3, -5.3; **HRMS** (MALDI-TOF) m/z [M + Na]$^+$ calcd for $C_{23}H_{41}NO_5NaSiS$ 494.2367, found 494.2366.

14:



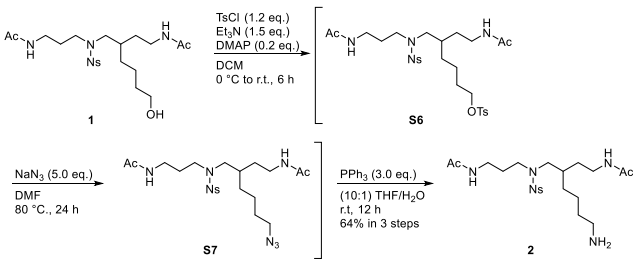
To a solution of **13** (109.7 mg, 0.233 mmol, 1.0 eq.) and **5** (77.1 mg, 0.256 mmol, 1.1 eq.) in DMF (1.0 mL) was added Cs_2CO_3 (113.7 mg, 0.349 mmol, 1.5 eq.) at room temperature, and the mixture was stirred at 80 °C for 5 h. After cooling to room temperature, water and $CHCl_3$ were added. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined, and the solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30/1 to 20/1) to give **14** (116.2 mg, 0.193 mmol, 83%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95-7.93 (1H, m), 7.72-7.60 (3H, m), 6.46 (1H, brs), 6.01 (1H, brs), 3.58-3.55 (2H, m), 3.43-3.30 (2H, m), 3.26-3.12 (6H, m), 1.97 (3H, s), 1.97 (3H, s), 1.81-1.66 (3H, m), 1.52-1.41 (4H, m), 1.33-1.21 (4H, m), 0.87 (9H, s), 0.03 (6H, s); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.6, 170.4, 148.1, 133.6, 132.7, 131.7, 130.5, 124.1, 62.8, 52.8, 46.3, 36.9, 36.8, 34.0, 32.9, 31.1, 30.9, 28.0, 26.0, 23.3, 23.2, 22.5, 18.3, -5.3; **HRMS** (MALDI-TOF) m/z [M + Na]$^+$ calcd for $C_{27}H_{48}N_4O_7NaSiS$ 623.2905, found 623.2904.

1: To a solution of **14** (69.7 mg, 0.116 mmol, 1.0 equiv.) in THF (1.2 mL) was added *n*-Bu_4NF (1.0 M in THF, 0.35 mL, 0.35 mmol, 3.0 equiv.) in an ice bath, and the mixture was stirred at room temperature for 6 h. Then, water and $CHCl_3$ were added. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined, and the solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 4/1) to give **1** (51.9 mg, 0.107 mmol, 92%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89-7.87 (1H, m), 7.67-7.55 (3H, m), 6.58-6.55 (1H, m), 6.32-6.30 (1H, m), 3.55 (2H, t, J = 5.9 Hz), 3.36-3.08 (8H, m), 2.24 (1H, brs), 1.91 (6H, s), 1.73-1.62 (3H, m), 1.49-1.37 (4H, m), 1.34-1.18 (4H, m); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.9, 170.7, 148.1, 133.7, 132.6, 131.8, 130.5, 124.1, 62.0, 52.7, 46.2, 37.0, 36.7, 33.7, 32.4, 31.0, 30.4, 28.0, 23.2, 23.1, 22.2.; **HRMS** (MALDI-TOF) m/z [M + Na]$^+$ calcd for $C_{21}H_{34}N_4O_7NaS$ 509.2040, found 509.2042.

2:



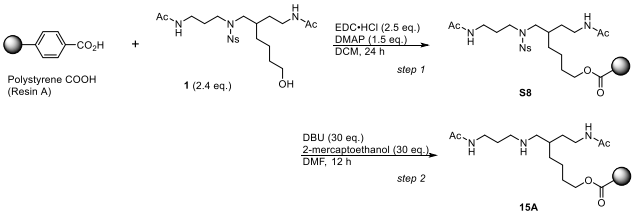
(Step 1): To a solution of **1** (187.9 mg, 0.386 mmol, 1.0 equiv.) in CH_2Cl_2 (2 mL) were added TsCl (88.3 mg, 0.463 mmol, 1.2 equiv.), Et_3N (80 µL, 0.589 mmol, 1.5 equiv.) and DMAP (9.4 mg, 0.077 mmol, 0.2 equiv.) in an ice bath, and the mixture was stirred at room temperature for 6 h. Then, water and $CHCl_3$ were added. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined, and the solution was concentrated under reduced pressure. The residue was passed through a short silica gel column chromatography ($CHCl_3$/MeOH = 15/1) to give the crude tosylated product **S6** (200.2 mg, 0.312 mmol).

(Step 2): To a solution of **S6** (200.2 mg, 0.312 mmol, 1.0 equiv.) in DMF (3 mL) was added NaN_3 (101.5 mg, 1.560 mmol, 5.0 equiv.) in an ice bath, and the mixture was stirred at room temperature for 6 h. Then, water and $CHCl_3$ were added. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined, and the solution was concentrated under reduced pressure. The residue was passed through a short silica gel column chromatography ($CHCl_3$/MeOH = 15/1) to give the crude azide product **S7** (145.4 mg, 0.284 mmol).

(Step 3): To a solution of **S7** (145.4 mg, 0.284 mmol, 1.0 equiv.) in THF (3 mL) and water (0.3 mL) was added PPh_3 (223.5 mg, 0.852 mmol, 3.0 equiv.) at room temperature, and the mixture was stirred for 12 h. Then, water and $CHCl_3$ were added. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined, and the solution was concentrated under reduced pressure. The residue was purified by C18-reversed phase silica gel column chromatography (CH_3CN/H_2O = 20/1) to give **2** (119.6 mg, 0.246 mmol, 64% for 3 steps) as a colorless oil.

^{1}H NMR (400 MHz, CD_3OD) δ 8.05-8.02 (1H, m), 7.82-7.74 (3H, m), 3.27-3.09 (6H, m), 2.64-2.61 (2H, m), 1.92 (6H, s), 1.76-1.67 (3H, m), 1.52-1.29 (8H, m); **^{13}C NMR** (100 MHz, CD_3OD) δ 173.3, 173.1, 149.6, 135.3, 133.7, 133.1, 131.6, 125.4, 53.1, 46.7, 42.2, 37.92, 37.89, 34.9, 33.8, 31.8, 31.7, 29.2, 24.2, 22.6 (2C); **HRMS** (MALDI-TOF) m/z [M + Na]$^+$ calcd for $C_{21}H_{35}N_5O_6NaS$ 508.2205, found 508.2200.

15A:

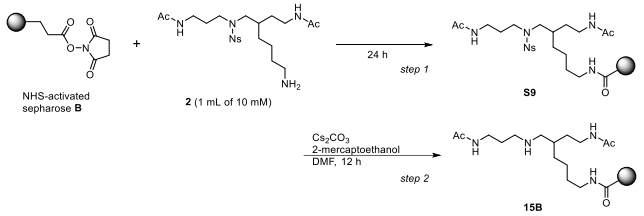


(Step 1): We referred to the literature method.[18] To a suspension of polystyrene COOH resin (Resin A: Watanabe Chemical Industries, LTD #A00262) (0.219 mmol/g, 100.0 mg, 0.219 mmol) in CH_2Cl_2 (1 mL) were added DiAcSpd analogue **1** (252.6 mg, 0.519 mmol, 2.4 eq.),

EDC·HCl (107.4 mg, 0.560 mmol, 2.6 eq.) and DMAP (40.1 mg, 0.328 mmol, 1.5 eq.) at r.t. After shaking at 1500 rpm by fast shaker (EYELA #CM-1000) for 24 h at r.t., the resin was then filtered, alternating washed 5 times with CH_2Cl_2, MeOH, EtOAc, Et_2O, H_2O, followed by CH_2Cl_2 and then dried in vacuo at 60 °C for 8 h to give the **S8** (199.8 mg). **IR** (ATR, cm^{-1}): 3288, 3069, 3027, 2924, 2853, 1707, 1654, 1648, 1638, 1543, 1492, 1450, 1369, 1344, 1274, 1161, 1107, 1019, 752, 698.

(Step 2): We referred to the literature method.[19] To a suspension of **S8** (~0.219 mmol/g, 100.0 mg, ~0.219 mmol) in DMF (1 mL) were added DBU (0.98 mL, 6.57 mmol, 30 eq.) and 2-mercaptoethanol (0.47 mL, 6.57 mmol, 30eq.) at r.t. After shaking at 1500 rpm by fast shaker for 12 h at r.t., the resin was then filtered, alternating washed 5 times with DMF, CH_2Cl_2, MeOH, EtOAc, Et_2O, H_2O, followed by CH_2Cl_2 and then dried in vacuo at 60 °C for 6 h to give the **15A** (71.9 mg, 0.0728 mmol, 37% loading). Kaiser test was positive. **IR** (ATR, cm^{-1}): 3286, 3064, 3024, 2924, 2852, 1707, 1641, 1544, 1535, 1492, 1450, 1369, 1273, 1180, 1107, 1028, 752, 698.

15B:



(Step 1): We referred to the product manual or literature method.[8, 20] 1 mL (~16-23 µmol) of NHS-activated sepharose 4 Fast Flow Lab Packs (Resin B: GE Healthcare Life Sciences # 17090601; 16-23 µmol/mL) in ^{i}PrOH was washed 3 times with 1 mL cold 1 mM HCl, the resin was then spin down and washed with 1 mL of coupling buffer (0.1 M $NaHCO_3$/ 0.5 M NaCl, pH8.4). To a suspension of resin was added 1 mL of 10 mM DiAcSpd analogue **2** at r.t. After shaking at 800 rpm by fast shaker for 24 h at r.t., the resin was then filtered, alternating washed 5 times with 1 mL of coupling buffer and 1 mL of acetate buffer (0.1 M CH_3COOH/CH_3COONa/0.5 M NaCl, pH4.1), followed by 3 times with 1 mL of MilliQ to give **S9**. **IR** (ATR, cm^{-1}): 3361, 2906, 1629, 1543, 1373, 1047, 966, 930, 891.

(Step 2): To a suspension of above **S9** in DMF (1 mL) were added Cs_2CO_3 (32.6 mg, 0.1 mmol) and 2-mercaptoethanol (7.0 µL, 0.1 mmol) at r.t. After shaking at 800 rpm by fast shaker for 12 h at r.t., the resin was then filtered, alternating washed 5 times with DMF, CH_2Cl_2, MeOH, EtOAc, Et_2O, H_2O, followed by alternating washed 5 times with 1 mL of coupling buffer and 1 mL of acetate buffer to give **15B**. Then the resin was washed 3 times with 1 mL of binding buffer (10 mM HEPES/ 100 mM KCl, 1 mM $MgCl_2$) and stored column in binding buffer. Use the suspension in SELEX without further purification. Since this resin could not be applied to dry *in vacuo*, the loading yield could not be calculated. The amine coupling is evaluated as progressing quantitatively as with other literature treatments. **IR** (ATR, cm^{-1}): 3342, 2911, 1629, 1369, 1049, 966, 929, 891.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported financially by JSPS Research Fellows from the JSPS (JP18J12822 to R. O.), a Grant-in-Aid for Scientific Research (JPT15KT00630, for Precisely Designed Catalysis with Customized Scaffolding, and JPT262880510 to M. A.) from JSPS and Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)) from AMED under Grant Number JP18am0101084, from JST ACT-C Grant Number JPMJCR12YM, Japan, and from AMED "Dynamic Alliance for Open Innovation Bridging Human, Environment and Materials" from the Ministry of Education, Culture, Sports, Science and Technology of Japan under Grant Number 17am0101084j0001. We would also like to thank Mr. R. F. B. Avena for his assistance in this manuscript.

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