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Short communication

Preparation of spermine conjugates with acidic retinoids with potent ribonuclease P inhibitory activity

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ABSTRACT

Novel mono- and diacylated spermines, readily obtained using isolable succinimidyl active esters of acidic retinoids for the selective acylation of free spermine or in situ activated acidic retinoids for acylating selectively protected spermine followed by deprotection, were shown to inhibit the ribozyme ribonuclease P more strongly than the parent retinoids.

1. Introduction

Retinoids constitute a large family of organic compounds structurally related to the naturally occurring Vitamin A and analogs, such as *all-trans*-retinoic acid (ATRA, **1**), and a variety of other synthetic analogs, such as acitretin (ACI, **2**) (Fig. 1). Retinoids can cause specific biological responses upon binding to and activating special receptors or groups of receptors. Natural and synthetic retinoids play an important role in vision, cell growth, reproduction, proliferation and differentiation of various epithelial or non-epithelial tissues. Although they are already widely used in systemic and topical treatment of various disorders, retinoids reveal a considerable number of side effects even when used in therapeutic doses. Thus, numerous new retinoid analogs have been synthesized in an attempt to improve the therapeutic index, biological profile and selectivity of these compounds for clinical application in dermatology, oncology, rheumatology and immunology [1–5]. The clinical application of synthetic retinoids in the management of recalcitrant and previously incurable neoplastic, inflammatory and keratinization disorders has introduced a real revolution in dermatology and other medical fields [6,7].

It has been recently reported that natural retinoids, like retinoic acid and retinol, as well as synthetic analogs of retinoic acid, e.g. isotretinoin (13-*cis*-retinoic acid), ACI and the arotinoids Ro 13-7410, Ro 15-0778, Ro 15-1570 and Ro 13-6298 but also other compounds, e.g. calcipotriol, anthralin and their combination, known for their antipsoriatic activity, inhibit the ribozyme ribonuclease P (RNase P) [8–12], which has been isolated and characterized from the slime mold *Dictyostelium discoideum* [13] and from normal human epidermal keratinocytes [14]. These findings advocate the hypothesis that retinoids, in addition to regulating DNA transcription, can also regulate the activity of enzymes playing key-roles in macromolecular biosynthesis, by their implication in post-transcriptional processes, in which binding to the retinoic acid receptors is not involved. RNase P is responsible for the ripening of the 5′ terminus of precursor tRNA molecules. RNase P activity has been found in almost every pro- and eukaryotic organisms studied so far [15]. An exception, concerning the archaeon *Nanoarchaeum equitans* has been published recently [16]. RNase P enzymes are complexes of RNA with proteins and their activity is mainly attributed to their RNA subunit. Several findings indicate that the structure of the RNA

Abbreviations: ATRA, *all-trans*-retinoic acid; ACI, acitretin; Ch, cyclohexyl; DCM, dichloromethane; SPM, spermine; RAR, retinoic acid receptor; RXR, retinoid X receptor; RNase P, ribonuclease P; RT, room temperature; TFA, trifluoroacetic acid.

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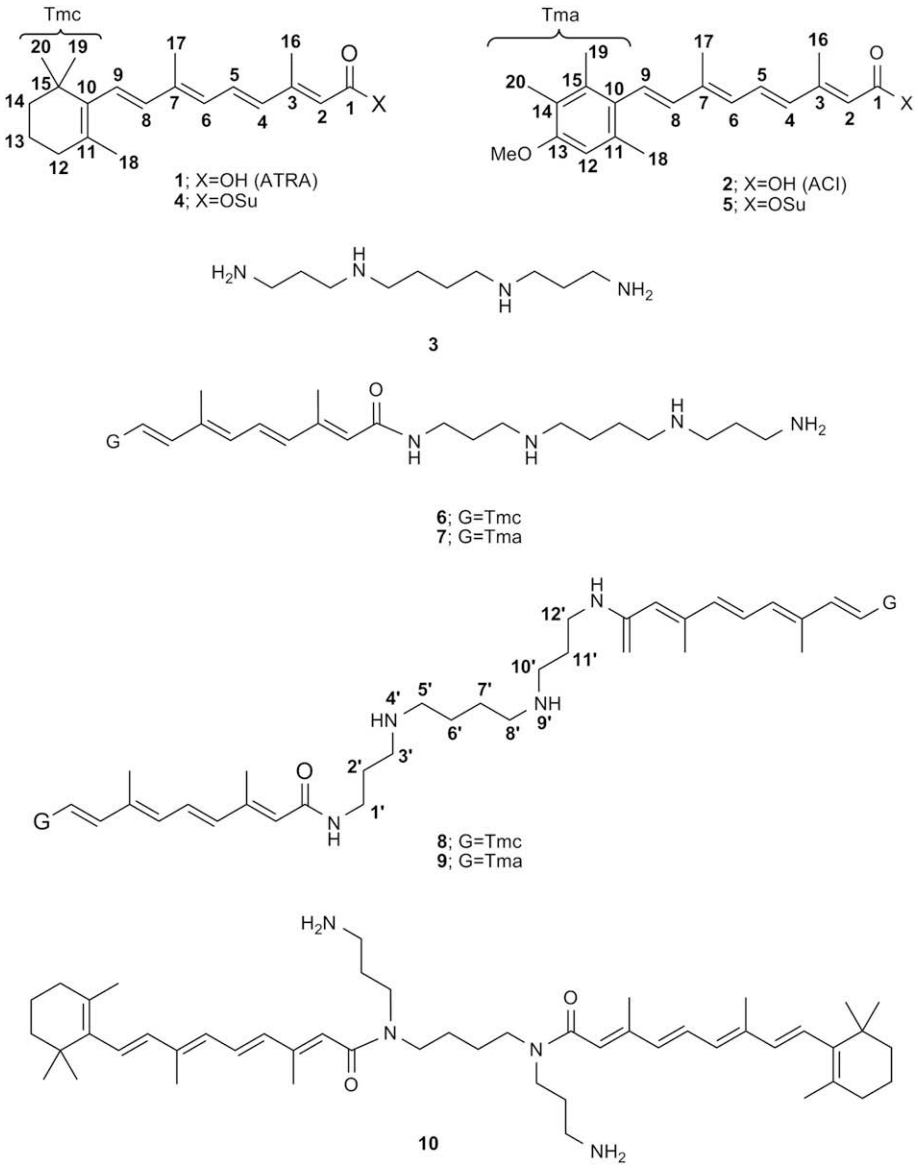
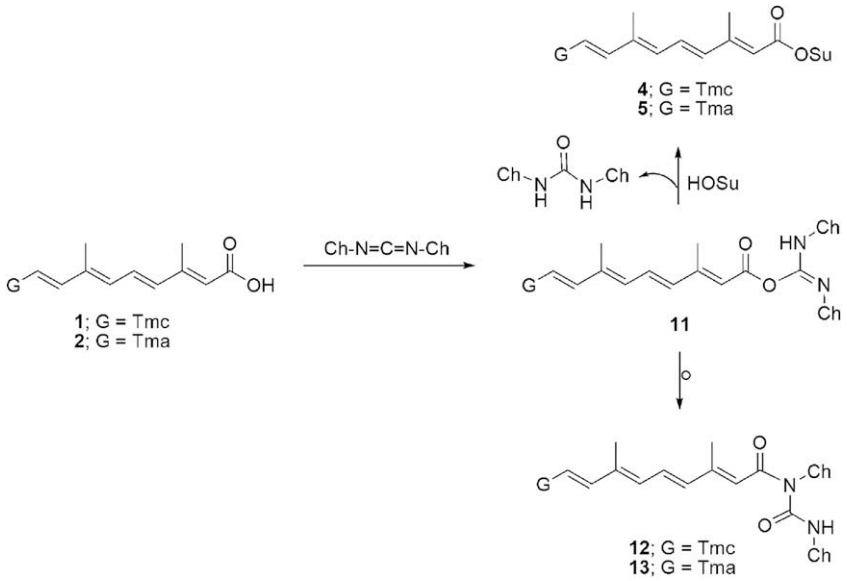


Fig. 1. Structures of compounds encountered in the present work.

subunit is of similar size in pro- and eukaryotic organisms and that the structures of RNase P from different eukaryotic organisms are similar. For these reasons, it appears that RNase P from *D. discoideum* and human epidermal keratinocytes are good models for the identification and development of new inhibitors.

On the other hand, linear polyamines, like spermine (SPM, **3**) and their compounds with other natural products, collectively coined as polyamine conjugates, are widely distributed in living organisms and exhibit interesting biological properties. In order to determine structure–biological activity relationships and possibly identify lead compounds for the development of polyamine-based pharmaceuticals, a variety of polyamine analogs and conjugates have been synthesized [17–20]. Due to their polycationic nature, polyamines interact strongly with nucleic acids and play an important role in their biosynthesis and metabolism. They stabilize DNA conformation and can induce conformation changes through the formation of intra- or intermolecular bridges. Polyamines cause specific modifications of specialized RNA molecules, stabilize ribonucleases and stimulate the action of ribonucleases and ribozymes. They exert pleiotropic effects on protein synthesis, which are essential for normal growth and are involved in the differentiation processes of mammalian cells.

Attaching a polyamine on another bioorganic molecule results in the formation of a polyamine conjugate. Depending on the structure of the non-polyamine moiety, these conjugates are designed to exhibit improved biological activity on particular cellular targets or combine the activities of the constituent molecules. The conjugates **6**–**10** (Fig. 1) of the present work were synthesized in an attempt to combine the biological profiles of polyamines and retinoids and are suitable for structure–activity relationship studies. Thus, in conjugates **6** and **7** one molecule of an acidic retinoid (compound **1** or **2**) is attached at N-1 of SPM whereas in conjugates **8**–**10** two molecules of an acidic retinoid are attached to either the positions N-1 and N-12 (conjugates **8** and **9**) or the positions N-4 and N-9 of SPM (conjugate **10**). It was anticipated that the polyamine affinity for nucleic acids, e.g. the RNA part of RNase P, would enforce the binding of natural and synthetic retinoids on the same molecule. Recently, N^1,N^3-diretinoyl-1,3-diaminopropane was synthesized, from 1,3-diaminopropane and retinoyl chloride, as potential antitumour agent but showed low cytostatic activity [21].

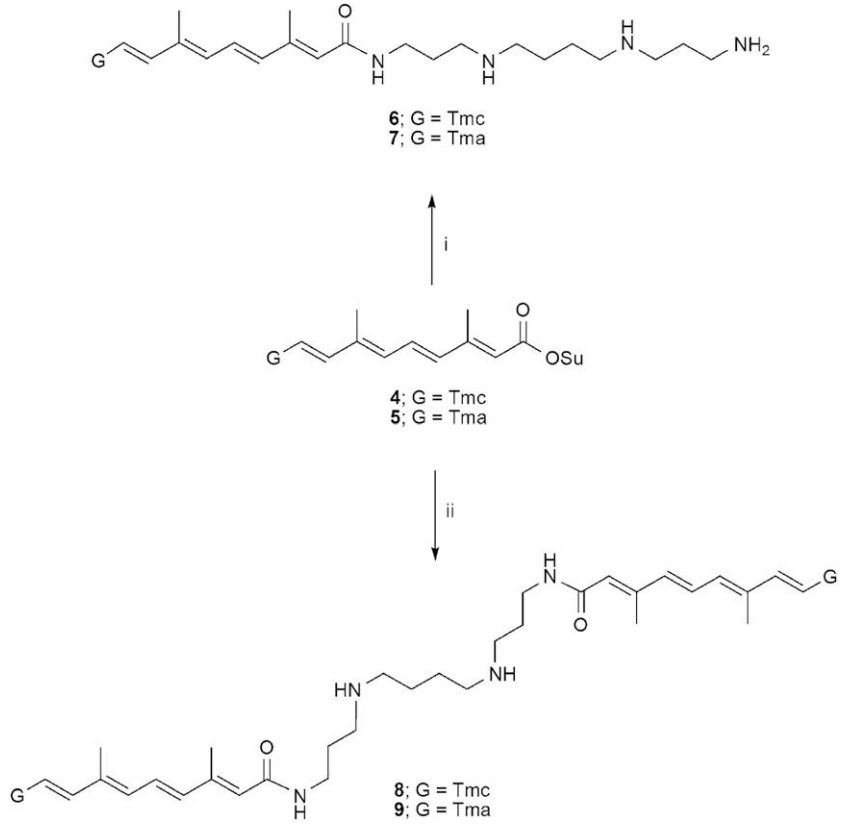


Scheme 1. Activation of acidic retinoids using HOSu in the presence of DCC. Reagents and conditions: HOSu/DCC, THF or DMF, 30 min at 0 °C then RT overnight, 81–85%.

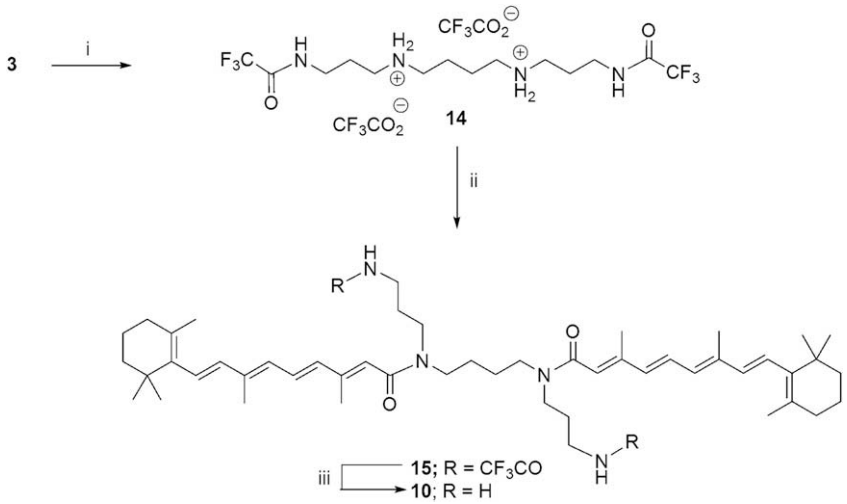
2. Results and discussion

2.1. Synthesis

Key-reaction in the synthesis of the SPM conjugates described in the present work is the coupling of an acidic retinoid or a suitably activated derivative with either free SPM (*direct method*) or suitably protected SPM (*indirect method*). The acidic retinoids used in this work were the commercially available *all-trans*-retinoic acid and acitretin. Taking into consideration the fact that the succinimidyl esters of cinnamic acids are hydrolytically stable, can be readily purified, if necessary, with flash column chromatography (FCC) and selectively acylate primary amino functions in the presence of secondary ones [22,23], we chose to activate the acidic retinoids also in the form of their corresponding 'active' esters with *N*-hydroxysuccinimide (HOSu). The required succinimidyl esters **4**



Scheme 2. Selective mono- and diacylation of SPM by isolated syccinimidyl esters of acidic retinoids. Reagents and conditions: (i) [4/5:SPM = 1:3], 0 °C to RT, DCM, 41–45%; (ii) [4/5:SPM = 2:1], 0 °C, 1 h, DCM, 78–80%.



Scheme 3. Indirect N^4,N^9-diacylation of SPM. Reagents and conditions: (i) CF_3CO_2Et, MeCN, overnight reflux, 93%; (ii) **1**/PyBrOP/$^iPr_2NEt$, 1.5 h, 0 °C to RT, 70%; (iii) 5 N aq. NaOH/MeOH, 2 h, RT, 67%.

and **5** were simply obtained (Scheme 1) by treating the acidic retinoids **1** and **2**, respectively, with HOSu in the presence of the coupling agent *N*,*N*′-dicyclohexylcarbodiimide (DCC) together with small quantities of a less polar byproduct, which was shown by ESI-MS to be the rearranged compounds (**12** and **13**, respectively) of the initial adduct **11** of the retinoids with DCC. The succinimidyl esters **4** and **5** could be readily obtained pure in 81–85% yields, following purification by FCC. When crude esters **4** and **5** were directly reacted with SPM, the aforementioned byproducts of the activation reaction remained in the reaction solvent, as expected, unchanged. Formation of these *N*-acylurea byproducts could be completely suppressed by using a larger excess of HOSu (e.g. acidic retinoid:HOSu = 1:2). However, under these conditions, the activation rate was significantly lower and additional portions of DCC were necessary to drive the reaction to completion.

Esters **4** and **5** were then used to acylate SPM. Indeed, direct reaction of active esters **4** and **5** with SPM (in the molar ratio 2:1) produced the diacylated SPM derivatives **8** and **9** (Scheme 2) which precipitated out from the reaction mixture as their corresponding bishydroxysuccinimidate salts. These compounds were thus obtained pure in 78–80% yields, by simple filtration. We then turned our attention to the selective monoacylation of SPM on its primary amino functions. Although an indirect multi-step preparation of the desired compounds **6** and **7** in rather low yields has been already reported [24], we considered of interest to examine the possibility of more direct methods. Indeed, direct reaction of an excess of SPM with active esters **4** and **5** (ratio of SPM to active ester = 3:1), followed by RP-HPLC purification of the reaction mixture thus obtained, afforded the requested compounds **6** and **7**, respectively, as the corresponding tristrifluoroacetate salts in 41–45% yields (Scheme 2).

The preparation of SPM acylated at its secondary amino functions by acidic retinoids is exemplified in Scheme 3 with the preparation of the SPM conjugate **10**. Thus, selective trifluoroacetylation of the primary amino functions of SPM with CF_3CO_2Et gave SPM derivative **14** [25,26]. The latter was then acylated on the remaining free secondary amino functions with ATRA in the presence of the powerful coupling agent bromotripyrrolidinophosphonium hexafluorophosphate (PyBrOP) and $^iPr_2NEt$, to afford the fully protected SPM derivative **15**. From this compound, the projected conjugate **10** was readily obtained through routine deprotection of the primary amino function by alkaline hydrolysis.

2.2. Effect of spermine conjugates with acidic retinoids on RNase P activity

We have developed a method through which we can estimate rapidly the biological activity of the spermine–acidic retinoid conjugates. This method is based on the effect of the polyamine–retinoid conjugates on RNase P activity and is described in detail in Section 4. Due to the high hydrophobicity of the conjugates, all

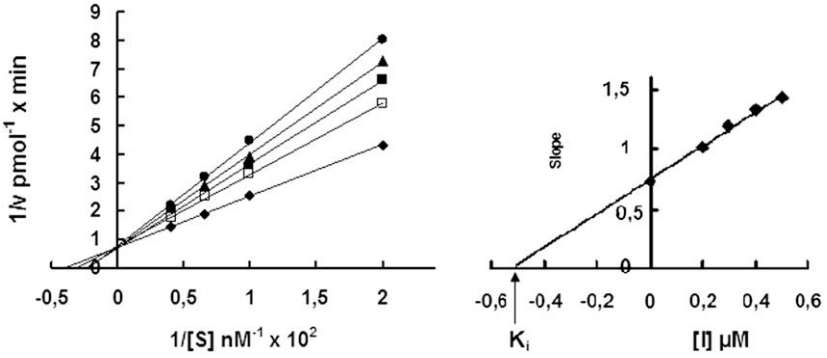


Fig. 2. Double reciprocal plot (1/*v* versus 1/[pre-tRNA]) for RNase P reaction in the presence of conjugate **8**. The reaction was carried out at the indicated concentrations in the presence or absence of inhibitor. All reactions were carried out at 37 °C in 20 μL buffer D in the presence of 10% DMSO. (◆) Without inhibitor, with conjugate **8** at (□) 2 μM, (■) 3 μM, (▲) 4 μM, (●) 5 μM. Right panel: Replot of the slopes of the double reciprocal lines versus inhibitor (I) concentrations.

Table 1
Equilibrium constants derived from primary and secondary plots.[a]

Retinoids	K_i (μM)	Spermine conjugates with acidic retinoids	K_i (μM)
Retinol	1475[b]	SPM–ATRA conjugate **6**	2.4
ATRA	15	SPM–ATRA conjugate **8**	0.5
Isotretinoin	20[b]	SPM–ATRA conjugate **10**	1.1
ACI	8	SPM–ACI conjugate **7**	2.45
Ro 13-7410	45[c]	SPM–ACI conjugate **9**	0.95
Ro 15-0788	2800[c]		
Ro 15-1570	3600[c]		
Ro 13-6298	4350[c]		
ATRA + SPM	15		

[a] The K_i values are calculated from the negative intercept of the slope replots (see Fig. 2).
[b] Ref. [8].
[c] Ref. [9].

assays were carried out in the presence of 10% DMSO. At this concentration, DMSO marginally affects (K_i = 5 M) the catalytic parameters of *D. discoideum* RNase P [8]. All synthesized conjugates exert distinct inhibitory effects on *D. discoideum* RNase P activity. The type of this inhibition was elucidated by a detailed kinetic analysis. In order to carry out such analysis, the initial velocity in the presence or absence of the conjugate was determined from the initial slopes of time plots (not shown). The data were plotted in double reciprocal plots ($1/v$ versus $1/[S]$) with increasing concentrations of the conjugates and the K_i values (the dissociation constant of the inhibitor (I), where I is a spermine–acidic retinoid conjugate, with the enzyme) were determined from the replots of the slopes of the double reciprocal plots versus the inhibitor's concentration. These replots lead to the graphical determination of K_i values from the negative intercept of the line with the I-axis. Fig. 2 shows the double reciprocal plot and the slope replot (right panel) for conjugate **8**. The linearity of the slope replot is indicative of simple competitive inhibition and leads to the graphical determination of K_i = 0.5 μM. Similar plots were constructed for all conjugates. This analysis showed that the type of inhibition of all conjugates is simple competitive. According to this finding, we could assign the potency of conjugates solely on the basis of the K_i value, which is a very good measure for the accurate potency of the spermine–acidic retinoid conjugates. The K_i values of the effect of the conjugates on *D. discoideum* RNase P activity are presented in Table 1 in comparison to known natural and synthetic retinoids [8] and arotinoids [9]. Thus, among the conjugates **6**–**10**, the conjugate **8** appears to be the strongest inhibitor. Notably, conjugate **8** is 90, 40, 30 and 16 times more potent than Ro 13-7410, isotretinoin, ATRA and ACI, respectively. Also, double acylation of SPM (conjugates **8** and **9**) seems to result in stronger inhibition than single acylation (conjugates **6** and **7**), whereas double acylation of primary amino functions (e.g. conjugate **8**) is more effective than double acylation of the secondary ones (conjugate **10**). Finally, SPM conjugate **8** is a more effective inhibitor than a combination of the unconjugated molecules ATRA and SPM.

3. Conclusion

Spermine conjugates (amides) with acidic retinoids, like ATRA and ACI, were for the first time prepared through the condensation of either free or selectively protected SPM with the succinimidyl active esters of the retinoids or with the free acids in the presence of the coupling agent PyBrOP, followed by deprotection. These conjugates were invariably stronger inhibitors of the enzyme RNase P, isolated from the slime mold *D. discoideum*, than the parent compounds. The most potent inhibitor in this series was the diacylated derivative of SPM at the external (primary) amino functions. Diacylation of the internal (secondary) amino functions and monoacylation seem to lead to compounds presenting weaker inhibition. The synthesis and the biological evaluation of additional conjugates of ATRA and ACI, as well as other acidic retinoids, with other polyamines are currently in progress to determine the effect of the nature of the acidic retinoid and of the polyamines on the inhibitory activity of these conjugates on RNase P.

4. Experimental section

4.1. General

Capillary melting points were taken on a Büchi SMP-20 apparatus and are uncorrected. IR spectra were recorded as KBr pellets, unless otherwise stated, on a Perkin–Elmer 16PC FT-IR spectrophotometer. ^{1}H NMR spectra were obtained at 400.13 MHz and ^{13}C NMR at 100.62 MHz on a Bruker Avance 400 DPX spectrometer at ambient temperature with the exception of compounds **8** and **9** which were recorded at 70 °C. Electron-Spray Ionization (ESI) mass spectra were obtained on a Micromass-Platform LC spectrometer for solutions of the measured compounds in MeOH. The high resolution MS (HRMS) experiments were carried out in a hybrid Q-Star Pulsar-i (MDS Sciex Applied Biosystems, Toronto, Canada) mass spectrometer equipped with an ion spray ionization source. Microanalyses were performed on a Carlo Erba EA 1108 Elemental Analyzer. Preparative HPLC was performed on a Waters system equipped with a 600E system controller using a Lichrosorb RP-18 reversed phase preparative column (30 × 300 mm) with a 10 μm Bondapak C18 packing material. Separations were achieved with a stepped linear gradient of acetonitrile (containing 0.08% TFA) in water (containing 0.08% TFA) over 60 min at a flow rate of 9 mL/min. Elution of the compounds was determined from the absorbances at 254 and 230 nm (Waters 996 Photodiode Array Detector). Compound purity was assessed by analytical HPLC (Nucleosil-120 C18, 250 × 4.00 mm) with a linear gradient of 20–100% acetonitrile (containing 0.08% TFA) in water (containing 0.08% TFA) over 30 min at a flow rate of 1 mL/min. Lyophilization of the pure products was performed by a Labconco Freezone 4.5 system. Flash Column Chromatography (FCC) was performed on Merck silica gel 60 (230–400 mesh) and Thin Layer Chromatography (TLC) on Merck silica gel F_{254} films (0.2 mm) precoated on aluminium foil. The solvent systems used were: (A) PhMe/EtOAc (95:5), (B) PhMe/EtOAc (9:1), (C) PhMe/EtOAc (1:1), (D) $CHCl_3$/MeOH/conc. NH_3 (6:4:0.4), (E) $CHCl_3$/MeOH/conc. NH_3 (5:5:0.5). Spots were visualized with UV light at 254 nm and ninhydrin (where applicable). All solvents used were dried according to standard procedures prior to use. Experiments involving retinoids were routinely conducted under an atmosphere of Ar and with protection from light. Drying of solutions was effected with anhydrous Na_2SO_4, whereas evaporation of the solvents was performed under reduced pressure in a rotary evaporator at a bath temperature not exceeding 40 °C.

4.2. General method for the preparation of succinimidyl esters of acidic retinoids

To an ice-cold solution of acidic retinoid **1** or **2** (10 mmol) in dry THF or DMF (30 mL) were added sequentially HOSu (1.73 g, 15 mmol) and DCC (2.48 g, 12 mmol) and the resulting mixture was stirred for an additional 30 min at 0 °C and for overnight at ambient temperature. The precipitated DCU was filtered off and washed several times with EtOAc. The combined filtrates were washed sequentially with an ice-cold 5% aqueous (aq.) $NaHCO_3$ solution, H_2O and twice with brine. Drying, followed by filtration and evaporation of the solvent left a residue. FCC of the residue followed by evaporation of the eluant and trituration with Et_2O gave the succinimidyl esters as yellowish crystalline compounds. The

byproducts **12** and **13** of the reactions were also isolated in 6–7% yields as yellow solids and had R_f (A): 0.36, MS (ESI^+): *m*/*z* 555.54 (*M*Na), 533.58 (*M*H) (for **12**) and R_f (B): 0.44, MS (ESI^+): *m*/*z* 529.44 (*M*Na), 507.53 (*M*H) (for **13**).

4.2.1. *Succinimidyl* all-trans-*retinoate* (***4***)

Yield: 3.38 g (85%), R_f (A): 0.24, m.p.: 173–76 °C, MS (ESI^+): *m*/*z* 833.20 (2*M*K), 818.24 (2*M*Na), 436.07 (*M*K), 420.04 (*M*Na), 398.09 (*M*H), 283.08 (*M*-$C_4H_4NO_3$), IR (KBr): cm^{-1} 1758, 1732, 1626, 1595, 1574, 1558, ^1H NMR ($CDCl_3$): δ 7.13 (1H, dd, *J* 12 and 16 Hz, H-5), 6.33 (2H, d, *J* 16 Hz, H-4 and H-8), 6.17 (1H, d, *J* 12 Hz, H-6), 6.16 (1H, d, *J* 16 Hz, H-9), 5.95 (1H, s, H-1), 2.85 (4H, br s, COCH_2CH_2CO), 2.38 (3H, s, H-16), 2.02 (5H, br s, H-17 and H-12), 1.72 (3H, s, H-18), 1.66–1.57 (2H, m, H-13), 1.50–1.44 (2H, m, H-14), 1.01 (6H, s, H-19 and H-20) ppm, ^{13}C NMR ($CDCl_3$): δ 169.8 (*C*OCH$_2$CH$_2$*C*O), 161.7 (C-1), 160.1 (C-3), 142.0 (C-7), 137.8 (C-10), 137.2 (C-5), 134.0 (C-4), 133.9 (C-8), 130.7 (C-11), 130.1 (C-6), 129.3 (C-9), 110.8 (C-2), 39.8 (C-14), 34.4 (C-15), 33.3 (C-12), 29.1 (C-19, C-20), 25.8 (COCH$_2$*C*H$_2$CO), 21.9 (C-18), 19.4 (C-13), 14.8 (C-16), 13.2 (C-17) ppm, Anal. Calcd. for $C_{24}H_{31}NO_4$: C, 72.52; H, 7.86; N, 3.52. Found: C, 72.69; H, 7.72; N, 3.28.

4.2.2. *Succinimidyl acitretinate* (***5***)

Yield: 3.43 g (81%), R_f (B): 0.31, m.p.: 177–79 °C, MS (ESI^+): *m*/*z* 869.25 (2*M*Na), 462.04 (*M*K), 446.33 (*M*Na), 424.04 (*M*H), 309.11 (*M*-$C_4H_4NO_3$), IR (KBr): cm^{-1} 1751, 1736, 1638, 1590, 1573, 1561, ^1H NMR($CDCl_3$): δ 7.18 (1H, dd, *J* 12 and 16 Hz, H-5), 6.76 (1H, d, *J* 16 Hz, H-4), 6.62 (1H, s, H-12), 6.38 (1H, d, *J* 16 Hz, H-8), 6.25 (1H, d, *J* 16 Hz, H-9), 6.23 (1H, d, *J* 12 Hz, H-6), 5.97 (1H, s, H-2), 3.82 (3H, s, OCH_3), 2.83 (4H, br s, COCH_2CH_2CO), 2.40 (3H, s, H-16), 2.31 (3H, s, H-18), 2.25 (3H, s, H-19), 2.16 (3H, s, H-20), 2.14 (3H, s, H-17) ppm, ^{13}C NMR ($CDCl_3$): δ 169.7 (*C*OCH$_2$CH$_2$*C*O), 161.6 (C-1), 159.8 (C-3), 156.3 (C-13), 141.5 (C-7), 137.9, 134.3, 133.7, 129.9, 129.8 (C-4, C-5, C-6, C-8, C-9), 136.0, 134.0, 129.6, 128.2 (C-10, C-11, C-14, C-15), 110.9, 110.0 (C-2, C-12), 55.5 (OCH_3), 25.7 (COCH$_2$*C*H$_2$CO), 21.4 (C-16), 17.5 (C-18), 14.7, 13.1 (C-19, C-20), 11.8 (C-17) ppm, Anal. Calcd for $C_{25}H_{29}NO_5$: C, 70.90; H, 6.90; N, 3.31. Found: C, 70.60; H, 7.05; N, 3.12.

4.3. *General method for the preparation of* N^1,N^{12}-*bisacylated spermines*

To an ice-cold solution of **3** (0.55 g, 2.7 mmol) in dry DCM (25 mL) was added ester **4** or **5** (5 mmol). The resulting solution was stirred for an additional hour at 0 °C and then allowed to reach ambient temperature. The precipitated product was filtered off, washed on the filter with DCM and dried under reduced pressure to give pure product in the form of the corresponding bishydroxysuccinimidate salt.

4.3.1. N^1,N^{12}-*Bis*(all-trans-*retinoyl)spermine* (***8***)

Yield: 2.15 g (80%), yellow powder, R_f (D): 0.40 (free base) and 0.13 (HOSu), m.p.: 149–52 °C, IR (KBr): cm^{-1} 3430, 3317, 1674, 1647, MS (ESI^+): *m*/*z* 790.25 (*M*Na) 768.34 (*M*H), 767.34 (*M*), ^1H NMR (d_6-DMSO): δ 7.75 (2H, unresolved t, N*H*CO), 6.88 (2H, dd, *J* 12 and 16 Hz, H-5), 6.27 (2H, d, *J* 16 Hz, H-4), 6.23 (2H, d, *J* 16 Hz, H-8), 6.17 (2H, d, *J* 12 Hz, H-6), 6.13 (2H, d, *J* 16 Hz, H-9), 5.83 (2H, s, H-2), 5.69 (4H, s, H-4′ and H-9′), 3.18–3.09 (4H, unresolved q, H-1′ and H-12′), 2.61–2.44 (16H, m, H-3′, H-5′, H-8′, H-10′, COCH_2CH_2CO), 2.25 (6H, s, H-16), 2.01 (4H, unresolved t, H-12), 1.95 (6H, s, H-17), 1.68 (6H, s, H-18), 1.63–1.53 (8H, unresolved quintet, H-13, H-2′, H-11′), 1.48–1.39 (8H, m, H-14, H-6′, H-7′), 1.02 (12H, s, H-19 and H-20) ppm, ^{13}C NMR (d_6-DMSO): δ 173.8 (*C*OCH$_2$CH$_2$*C*O), 166.9 (C-1), 146.6 (C-3), 138.2, 137.9, 137.7, 136.9 (C-5, C-7, C-10, C-11), 130.7, 129.8, 129.5, 127.8 (C-4, C-6, C-8, C-9), 123.5 (C-2), 48.8 (C-3′, C-10′), 46.6 (C-5′, C-8′), 40.8–39.5 (C-14, solvent), 36.9 (C-1′, C-12′), 34.5 (C-15), 33.2 (C-12), 29.4 (C-19, C-20), 28.9 (C-2′, C-11′), 26.7 (C-6′, C-7′), 25.8 (COCH_2CH_2CO), 22.1 (C-18), 19.4 (C-13), 13.7, 13.2 (C-16, C-17) ppm, Anal. Calcd. for $C_{58}H_{88}N_6O_8$: C, 69.85; H, 8.89; N, 8.43. Found: C, 69.56; H, 8.97; N, 8.62. Found: C, 69.56; H, 8.97; N, 8.62. HRMS ($C_{50}H_{78}N_4O_2 + H^+$) calcd.: 767.6198. Found: 767.6248.

4.3.2. N^1,N^{12}-*Bisacitretinylspermine* (***9***)

Yield: 2.21 g (78%), yellow powder, R_f (D): 0.45 (free base) and 0.13 (HOSu), m.p.: 167–69 °C, IR (KBr): cm^{-1} 3486, 3274, 1672, 1648, MS (ESI^+): *m*/*z* 820.41 (*M*H), 819.39 (*M*), 589.80 (*M*-$C_{36}H_{54}N_4O_3$), 410.41 (*M*H/2), ^1H NMR ($CDCl_3$): δ 8.00 (2H, unresolved t, N*H*CO), 6.93 (2H, dd, *J* 12 and 16 Hz, H-5), 6.68 (2H, s, H-12), 6.66 (2H, d, *J* 16 Hz, H-4), 6.31 (2H, d, *J* 16 Hz, H-8), 6.30 (2H, d, *J* 16 Hz, H-9), 6.26 (2H, d, *J* 12 Hz, H-6), 5.82 (2H, s, H-2), 3.75 (6H, s, OCH_3), 3.16–3.04 (4H, unresolved q, H-1′ and H-12′), 2.65–2.52 (8H, m, H-3′, H-5′, H-8′, H-10′), 2.27 (6H, s, H-16), 2.24 (6H, s, H-18), 2.17 (6H, s, H-19), 2.05 (12H, s, H-17 and H-20), 1.61–1.48 (8H, m, H-2′ and H-11′), 1.47–1.36 (4H, unresolved m, H-6′ and H-7′) ppm, ^{13}C NMR ($CDCl_3$): δ 173.85, 173.83 (*C*OCH$_2$CH$_2$*C*O, C-1), 156.3 (C-3), 156.2 (C-13), 138.3, 138.2, 137.2, 135.7, 134.1, 131.3, 130.0, 129.5, 128.1 (C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-15), 123.6, 122.2 (C-12, C-14), 110.7 (C-2), 55.9 (OCH_3), 48.5 (C-3′, C-10′), 44.4 (C-5′, C-8′), 36.8 (C-1′, C-12′), 28.7 (C-2′, C-11′), 26.4 (C-6′, C-7′), 25.8 (COCH$_2$CH$_2$CO), 21.8 (C-16), 17.8 (C-18), 15.8 (C-19), 13.2 (C-20), 12.4 (C-17) ppm, Anal. Calcd. for $C_{60}H_{84}N_6O_{10}$: C, 68.68; H, 8.07; N, 8.01. Found: C, 68.94; H, 7.85; N, 7.76. HRMS ($C_{52}H_{74}N_4O_4 + H^+$) calcd.: 819.5783. Found: 819.5831.

4.4. *General direct method for the preparation of* N^1-*monoacylated spermines*

To an ice-cold solution of SPM (0.21 g, 1.05 mmol) in DCM (10 mL) a solution of active ester **4** or **5** (0.35 mmol) in DCM (10 mL) was added dropwise over 1 h. The resulting reaction mixture was stirred at ambient temperature until the consumption of active ester (monitored by TLC). The reaction mixture was diluted with DCM and washed sequentially with an ice-cold 5% aqueous (aq.) $NaHCO_3$ and brine. Drying over Na_2SO_4, followed by filtration and evaporation of the solvent left an oily residue from which pure products **6** and **7** were obtained by preparative RP-HPLC as the corresponding tristrifluoroacetate salt.

4.4.1. N^1-*(*All-trans-*retinoyl)spermine* (***6***)

Yield: 0.13 g (45%), yellow foam, t_R: 16.93 min, IR (KBr): cm^{-1} 3422, 3046, 2928, 1676, 1646, MS (ESI^+): *m*/*z* 485.36 (*M*H), ^1H NMR (d_6-DMSO): δ 8.87 (2H, br s, H-4′), 8.69 (2H, br s, H-9′), 8.18 (1H, t, *J* 8 Hz, N*H*CO), 8.06 (3H, br s, NH_3^+), 6.93 (1H, dd, *J* 12 and 16 Hz, H-5), 6.31 (1H, d, *J* 16 Hz, H-4), 6.26 (1H, d, *J* 12 Hz, H-6), 6.20 (1H, d, *J* 16 Hz, H-8), 6.16 (1H, d, *J* 16 Hz, H-9), 5.83 (1H, s, H-2), 3.18 (2H, q, *J* 8 Hz, H-1′), 3.05–2.81 (10H, two unresolved m, H-3′, H-5′, H-8′, H-10′, H-12′), 2.28 (3H, s, H-16), 1.96 (2H, unresolved t, H-12), 1.91 (3H, s, H-17), 1.89 (2H, quintet, *J* 7 Hz, H-2′), 1.74 (2H, quintet, *J* 7 Hz, H-11′), 1.69 (3H, s, H-18), 1.64 (4H, unresolved m, H-6′ and H-7′), 1.60–1.55 (2H, m, H-13), 1.47–1.41 (2H, m, H-14), 1.01 (6H, s, H-19 and H-20) ppm, ^{13}C NMR (d_6-DMSO): δ 167.3 (C-1), 147.2 (C-3), 138.4, 137.9, 137.6, 136.7 (C-5, C-7, C-10, C-11), 130.6, 129.9, 129.8, 127.9 (C-4, C-6, C-8, C-9), 123.1 (C-2), 46.8 (C-5′, C-8′), 45.4 (C-10′), 44.5 (C-3′), 40.8–39.5 (C-14, solvent), 36.9 (C-12′), 36.2 (C-1′), 34.5 (C-15), 33.2 (C-12), 29.4 (C-19, C-20), 26.7 (C-11′), 24.4 (C-2′), 23.3, 23.2 (C-6′, C-7′), 22.0 (C-18), 19.3 (C-13), 13.8, 13.1 (C-16, C-17) ppm, HRMS ($C_{30}H_{52}N_4O + H^+$) calcd.: 485.4214. Found: 485.4286.

4.4.2. N^1-*Acitretinylspermine* (***7***)

Yield: 0.12 g (41%), orange foam, t_R: 16.16 min, IR (neat): cm^{-1} 3428, 3046, 2941, 1678, 1636, MS (ESI^+): *m*/*z* 549.39 (*M*K), 511.46 (*M*H), 201.05 (*M*-SPM), 186.01 (*M*-$C_{10}H_{24}N_3$), 171.01 (*M*-$C_9H_{22}N_3$), ^1H NMR (d_6-DMSO): δ 8.92 (2H, br s, H-4′), 8.73 (2H, br s, H-9′), 8.26

(1H, t, *J* 8 Hz, N*H*CO), 8.06 (3H, br s, NH_3^+), 7.03 (1H, dd, *J* 12 and 16 Hz), 6.76 (1H, s, H-12), 6.75 (1H, d, *J* 16 Hz, H-4), 6.40 (1H, d *J* 16 Hz, H-8), 6.37 (1H, d, *J* 12 Hz, H-6), 6.34 (1H, d, *J* 16 Hz, H-9), 5.91 (1H, s, H-2), 3.82 (3H, s, OCH_3), 3.25 (2H, q, *J* 8 Hz, H-1′), 3.08–2.95 (10H, two unresolved m, H-3′, H-5′, H-8′, H-10′, H-12′), 2.36 (3H, s, H-16), 2.32 (3H, s, H-18), 2.25 (3H, s, H-19), 2.13 (6H, s, H-17 and H-20), 1.95 (2H, quintet, *J* 7 Hz, H-2′), 1.83 (2H, quintet, *J* 7 Hz, H-11′), 1.75–1.66 (4H, unresolved m, H-6′ and H-7′) ppm, ^{13}C NMR (d_6-DMSO): δ 166.7 (C-1), 156.1 (C-3), 156.0 (C-13), 138.1, 137.0, 135.6, 133.9, 131.1, 129.8, 129.3, 127.9 (C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11), 123.5, 122.1, 122.0 (C-12, C-14, C-15), 55.8 (OCH_3), 49.0, 48.2 (C-5′, C-8′), 46.8 (C-10′), 46.1 (C-3′), 37.1 (C-12′), 36.7 (C-1′), 28.4 (C-11′), 25.6 (C-2′, C-6′, C-7′), 21.5 (C-16), 17.6 (C-18), 13.6 (C-19), 13.0 (C-20), 12.2 (C-17) ppm, HRMS ($C_{31}H_{50}N_4O_2 + H^+$) calcd.: 511.4007. Found: 511.4076.

4.4.3. N^4,N^9-*Bis(*all-trans-*retinoyl)spermine (**10**)*

To an ice-cold solution of the bistrifluoroacetate salt of SPM derivative **14** (0.32 g, 0.5 mmol) in anhydrous $CHCl_3$ (1 mL) was added $^iPr_2NEt$ (0.7 mL, 4 mmol) and a mixture consisted of ATRA (0.3 g, 1 mmol) and PyBrOP (0.6 g, 1.28 mmol) in small portions over a period of 1 h. After 30 min at ambient temperature, the reaction mixture was diluted with $CHCl_3$ and washed sequentially with an ice-cold 5% aq. $NaHCO_3$ solution (twice) and water (twice). Drying and evaporation left a residue from which pure intermediate **15** was obtained through FCC, using as an eluant the solvent system C. Intermediate **15** (0.3 g, 0.31 mmol) was then dissolved in 4 mL MeOH and treated with 0.4 mL of a 5 N aq. NaOH solution for 2 h at ambient temperature. MeOH was then removed under reduced pressure and the residue was taken up in 20 mL water and extracted twice with DCM. The combined organic layers were washed twice with brine, dried, evaporated and subjected to FCC using as an eluant the solvent system E. Fractions containing the pure conjugate **10** were pooled, washed with water and evaporated under vacuo to afford the desired product.

4.4.4. N^4,N^9-*Bis(*all-trans-*retinoyl)*-N^1,N^{12}-*bistrifluoroacetylspermine (**15**)*

Yield: 0.34 g (70%), yellow foam, R_f(C): 0.13, IR (neat): cm^{-1} 3267, 2924, 1714, 1614, MS (ESI^+): *m*/*z* 998.90 (*M*K), 982.81 (*M*Na), 959.87 (*M*H), ^1H NMR ($CDCl_3$): δ 8.35 (2H, unresolved t, N*H*COCF$_3$), 6.83 (2H, dd, *J* 12 and 16 Hz, H-5), 6.21 (2H, d, *J* 16 Hz, H-4), 6.20 (2H, d, *J* 16 Hz, H-8), 6.11 (2H, d, *J* 12 Hz, H-6), 6.09 (2H, d, *J* 16 Hz, H-9), 5.81 (2H, s, H-2), 3.44–3.37 (4H, m, H-1′ and H-12′), 3.29–3.20 (8H, unresolved m, H-3′, H-5′, H-8′, H-10′), 2.09 (6H, s, H-16), 1.95 (4H, t, *J* 6 Hz, H-12), 1.92 (6H, s, H-17), 1.64 (6H, s, H-18), 1.56–1.48 (12H, m, H-13, H-2′, H-6′, H-7′, H-11′), 1.39–1.35 (4H, m, H-14), 0.98 (12H, s, H-19 and H-20) ppm, ^{13}C NMR ($CDCl_3$): δ 169.3 (C-1), 157.5 (q, *J* 36 Hz, *C*OCF$_3$), 147.7 (C-3), 139.2, 137.7, 137.2, 134.6, (C-5, C-7, C-10, C-11), 129.9, 129.2, 128.4, 127.3 (C-4, C-6, C-8, C-9), 119.2 (C-2), 116.1 (q, *J* 287 Hz, *C*F$_3$), 47.9 (C-1′, C-12′), 41.7 (C-3′, C-10′), 39.6 (C-14), 36.0 (C-5′, C-8′), 34.3 (C-15), 33.1 (C-12), 28.9 (C-19, C-20), 27.1 (C-6′, C-7′), 26.3 (C-2′, C-11′), 21.7 (C-18), 19.2 (C-13), 14.4 (C-16), 13.9 (C-17) ppm.

4.4.5. N^4,N^9-*Bis(*all-trans-*retinoyl)spermine (**10**)*

Yield: 0.16 g (67%), yellow foam, R_f(E): 0.26, IR (neat): cm^{-1} 3434, 1620, MS (ESI^+): *m*/*z* 807.74 (*M*K) 790.68 (*M*Na), 767.73 (*M*), ^1H NMR ($CDCl_3$): δ 6.78 and 6.74 (2H, two dd, *J* 12 and 16 Hz, H-5), 6.25 and 5.94 (2H, d, *J* 12 Hz, H-6), 6.22 (2H, d, *J* 16 Hz, H-4), 6.16 (2H, d, *J* 16 Hz, H-8), 6.10 (2H, s, H-2), 6.05 (2H, d, *J* 16 Hz, H-9), 3.42–3.24 (8H, m, H-3′, H-5′, H-8′, H-10′), 2.66–2.60 (4H, unresolved m, H-1′ and H-12′), 2.05 (6H, br s, H-16), 1.95 (4H, t, *J* 6 Hz, H-12), 1.91 (6H, s, H-17), 1.87–1.79 (4H, m, H-2′ and H-11′), 1.64 (6H, s, H-18), 1.53–1.44 (8H, m, H-13, H-6′, H-7′), 1.40–1.36 (4H, m, H-14), 0.95 (12H, s, H-19 and H-20) ppm, ^{13}C NMR ($CDCl_3$): δ 168.1 and 168.0 (C-1), 145.5 (C-3), 138.1, 137.7, 137.4 (C-7, C-10, C-11), 135.4 and 135.3 (C-5), 129.6, 128.8, 128.5, (C-6, C-8, C-9), 127.9 and 127.8 (C-4), 121.4 and 121.2 (C-2), 44.9 (C-3′, C-10′), 39.6 (C-14), 39.2 (C-1′, C-12′), 34.3 (C-15), 33.1 (C-12), 31.3 (C-5′, C-8′), 28.9 (C-19, C-20), 26.4 (C-6′, C-7′), 25.1 (C-2′, C-11′), 21.7 (C-18), 19.2 (C-13), 14.3 (C-16), 12.8 (C-17) ppm, HRMS ($C_{50}H_{78}N_4O_2 + H^+$) calcd.: 766.6125. Found: 766.6132.

4.5. Biological evaluation of conjugates as RNase P inhibitors

Growth of *D. discoideum* cells (strain A × 2 wild type), cell breakage, and purification of RNase P were essentially carried out as previously described [13]. RNase P assays were carried out at 37 °C in 20 μL buffer D (50 mM Tris/HCl pH 7.6, 10 mM NH_4Cl, 5 mM $MgCl_2$ and 5 mM dithiothreitol), containing 2–5 fmol pre-$tRNA^{ser}$ substrate (an in vitro labeled transcript of the *Schizosaccharomyces pombe* $tRNA^{ser}$ gene *supSI*) and 1.3 μg protein from the RNase P fraction. Stock solutions of conjugates were prepared in 100% dimethylosulfoxide (DMSO). Enzyme assays were carried out in the presence of 10% DMSO. The reactions were stopped by the addition of 5 μL stop dye (80% formamide, 50 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol). Reaction products were resolved on a denaturing 10% polyacrylamide/8M urea gel and visualized with phosphoimager Fujifilm FLA 3000. Gel bands were quantified using AIDA software.

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