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Suzuki–Miyaura Reaction in the Presence of *N*-Acetylcysteamine Thioesters Enables Rapid Synthesis of Biomimetic Polyketide Thioester Surrogates for Biosynthetic Studies

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Abstract: Biomimetic *N*-acetylcysteamine thioesters are essential for the study of polyketide synthases, non-ribosomal peptide synthetases and fatty acid synthases. The chemistry for their preparation is, however, limited by their specific functionalization and their susceptibility to undesired side reactions. Here we report a method for the rapid preparation of *N*-acetylcysteamine (SNAC) 7-hydroxy-2-enethioates, which are suitable for the study of various enzymatic domains of megasynthase enzymes. The method is based on a one-pot sequence of hydroboration and the Suzuki–Miyaura reaction. The optimization of the reaction conditions made it possible to suppress potential side reactions and to introduce the highly functionalized SNAC methacrylate unit in a high yield. The versatility of the sequence was demonstrated by the synthesis of the complex polyketide-SNAC thioesters **12** and **33**. Brown crotylation followed by the hydroboration to Suzuki–Miyaura reaction sequence enabled the introduction of the target motif in significantly fewer steps and with a higher overall yield and stereoselectivity than previously described approaches. This is the first report of a transition-metal-catalyzed cross-coupling reaction in the presence of an SNAC thioester.

Keywords: Suzuki–Miyaura reaction; biomimetic thioesters; polyketide synthases; enzymes; cyclases

Citation: Derra, S.; Schlotte, L.; Hahn, F. Suzuki–Miyaura Reaction in the Presence of *N*-Acetylcysteamine Thioesters Enables Rapid Synthesis of Biomimetic Polyketide Thioester Surrogates for Biosynthetic Studies. *Chemistry* **2023**, *5*, 1407–1418. https://doi.org/10.3390/chemistry5020096

Academic Editors: Christoph Janiak, Sascha Rohn and Georg Manolikakes

Received: 28 April 2023
Revised: 2 June 2023
Accepted: 2 June 2023
Published: 8 June 2023

1. Introduction

Thioesters are an important functional group in many biosynthetic systems. They often serve to link biosynthetic acyl intermediates to carrier thiols, which can be free molecules such as coenzyme A (CoA) or proteins. The important systems working with protein-bound metabolites are so-called megasynthase enzymes, such as fatty acid synthases, polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) and their hybrids [1,2]. They are responsible for the formation of polyketide and peptide natural products, including some of the most important small-molecule drugs in clinical use, such as erythromycin, rapamycin or epothilone. The availability of suitable substrate surrogates is essential for the functional study of such biosynthetic systems (Figure 1A). *N*-Acetylcysteamine (SNAC) thioesters are of particular importance for this as they effectively mimic the protein attachment of the substrate via the 4′-phosphopantetheine arm and thus allow simplified studies with active enzymes (Figure 1B) [3–10].

Acylated SNACs contain an acetamide and a thioester as conserved reactive functional groups, which afford them problematic properties (Figure 1C) [7,11]. The thioester can undergo side reactions with external or internal nucleophiles, resulting in the irreversible loss of substance. Due to its polarity, the acetamide can cause problems during substance purification and can, as a nucleophilic/protic group, cause undesired side reactions. The functionalization distance between the acetamide and thioester carries the risk that they act as a chelate ligand and interact with metals. The synthesis of the SNAC thioester surrogates of late-stage biosynthetic intermediates is as challenging as the synthesis of natural products of similar structural complexity but, for the above-mentioned reasons, has

the challenge of an additional problematic functional group. A useful strategy to overcome this problem would be to introduce the SNAC moiety at a late stage of synthesis along with a larger fraction of the polyketide moiety.

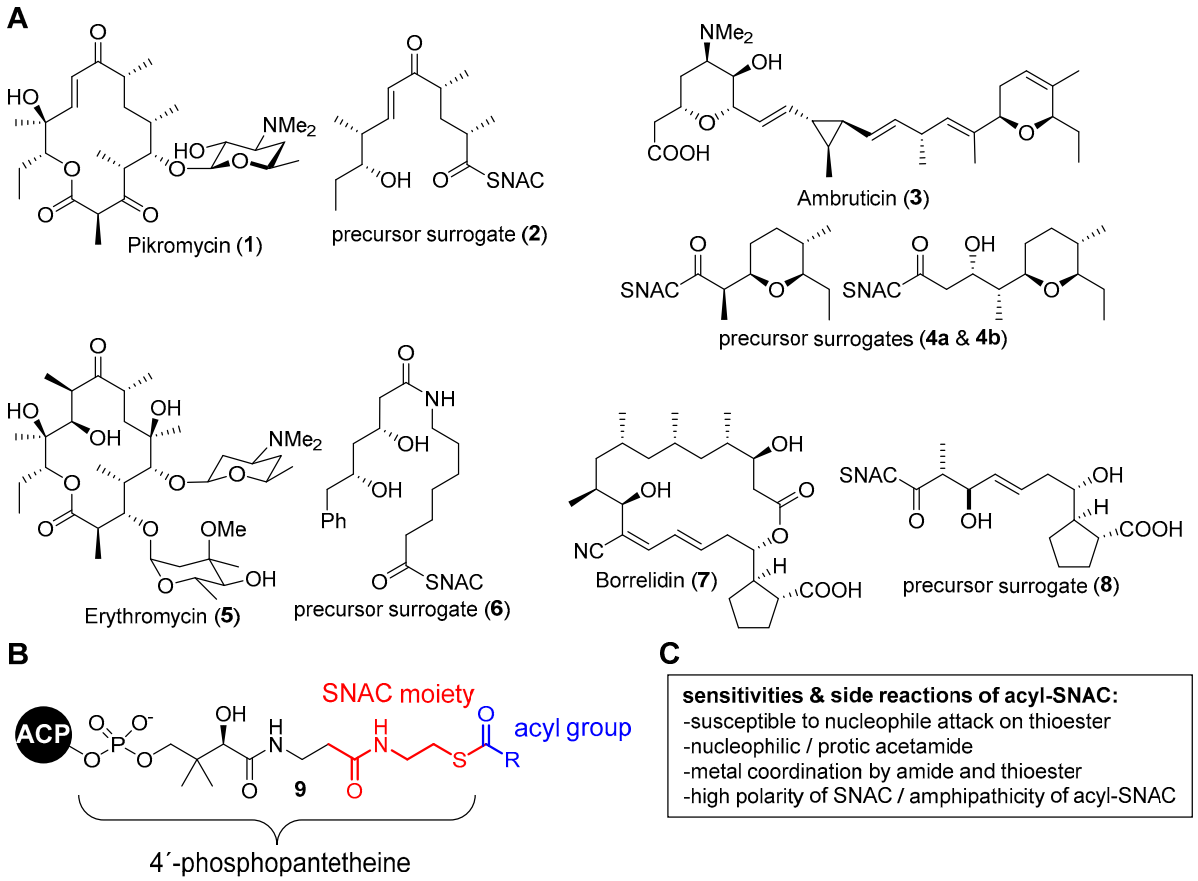


Figure 1. (**A**) Structure of the 4′-phosphopantetheine prosthetic group of acyl carrier proteins and the partial structure that is mimicked by SNAC. (**B**) Susceptibilities and side reactions of acyl-SNACs. (**C**) Prominent natural products and the structures of biomimetic SNAC thioesters that have been used for their biosynthesis studies.

Improving the specific methodology for the synthesis of complex polyketide–SNAC thioesters is therefore of great interest to the biosynthetic research community. Transition metal-mediated reactions are well suited to late-stage attachment in the convergent synthesis of complex biosynthetic thioester surrogates, but have only very rarely been described in the presence of SNAC thioesters. To the best of our knowledge, the literature currently only contains a report about olefin cross-metathesis between SNAC–acrylates and hydroxyolefins catalyzed by the second-generation Grubbs catalyst [12].

The Suzuki–Miyaura reaction (SMR) is a highly versatile Pd-catalyzed cross-coupling reaction. It allows couplings between halides and non-toxic boronic acid derivatives under relatively mild conditions (Figure 2) [13,14]. In addition to sp^2–sp^2 bond formations, it is now possible to carry out couplings between sp^2 and sp^3 centers, as well as between two sp^3 centers. Two aspects of the SMR could be problematic when applied to SNAC thioesters. On the one hand, the use of a base is necessary to accelerate the essential group transfer from the boronic acid to the Pd during the catalytic cycle (step 3). Moreover, Pd can also be inserted into the C–S bond of the thioester instead of the C–halide bond (step 1) [15]. This reactivity is so pronounced that it forms the basis of the Liebeskind–Srogl reaction, a modification of the SMR for the direct synthesis of ketones from thioesters [16,17].

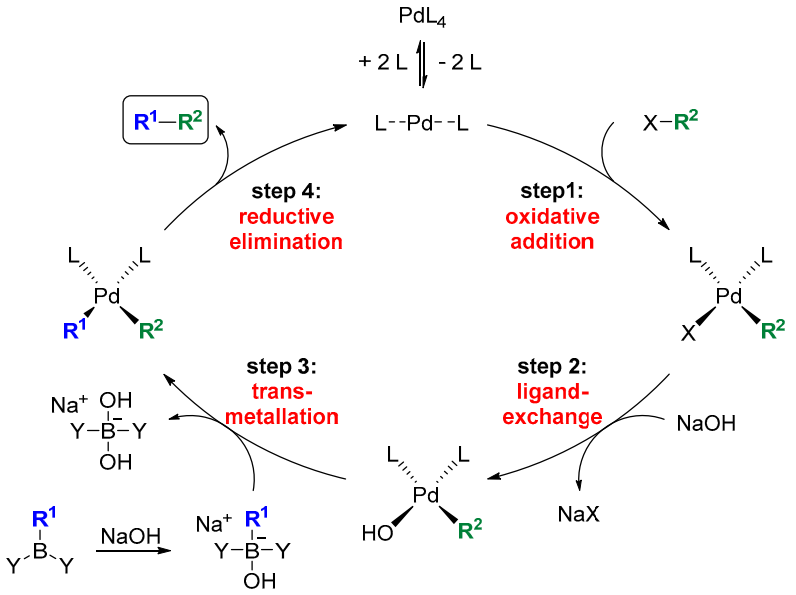


Figure 2. Mechanism of the SMR.

Among the diverse enzymatic PKS domains, cyclases that form saturated oxygen heterocycles via intramolecular oxa-Michael addition (IMOMA) stand out due to the synthetical value of this transformation (Figure 3A) [18,19]. It has been shown that they catalyze a ring formation with exceptional stereoselectivity and therefore represent a potential new type of biocatalyst [20–29]. For the study of such enzymes, SNAC-7-hydroxy-2-enethioates are required as substrate surrogates. The synthetic methodology used for the selective installation of this structural motif is, however, not well developed, making the generation of precursor libraries a difficult task. The multi-step routes described in the literature are either highly elaborate, are not stereoselective or lack flexibility, and are therefore narrow in their applicability [20–23]. For example, the synthesis of the SNAC surrogate of **10** in stereochemical pure form was accomplished in eight steps and required multiple purification procedures [21,22]. Furthermore, a lack of convergence makes it necessary to carry out the largest part of this sequence using different starter building blocks to access derivatives with variations in the eastern part of the molecule. Other reported routes are shorter, but also less flexible due to the choice of larger starting building blocks or the introduction reaction chosen for the SNAC thioester. Olefin cross-metathesis, for example, is only possible with SNAC–acrylthioates and not with SNAC–methacrylthioates. Therefore, we set out to develop a flexible, straightforward and broadly applicable method for the preparation of SNAC-7-hydroxy-2-enethioates.

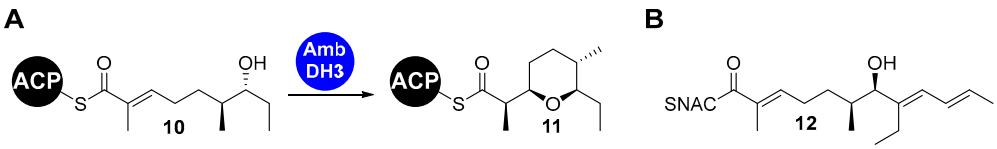


Figure 3. (**A**) IMOMA cyclases catalyze the intramolecular oxa-Michael addition to oxygen heterocycles. The natural reaction of AmbDH3 is shown as an example. (**B**) Structure of the target compound required for our biosynthetic studies.

As a solution, we turned to a sequence of hydroboration and SMR to assemble the backbone and directly introduce the SNAC moiety. The specific challenge was to effectively perform the SMR in the presence of the SNAC thioester, which has not been achieved before to the best of our knowledge. The versatility of the method should be shown on the example of the synthesis of **12** (Figure 3B). This compound was, on the one hand, specifically required for our enzymatic studies on new IMOMA cyclases (Figure 3A). On

the other hand, it represents a particularly challenging substrate during whose preparation various detrimental side reactions occur; it is thus a reasonable benchmark.

2. Materials and Methods

2.1. General Methods and Materials

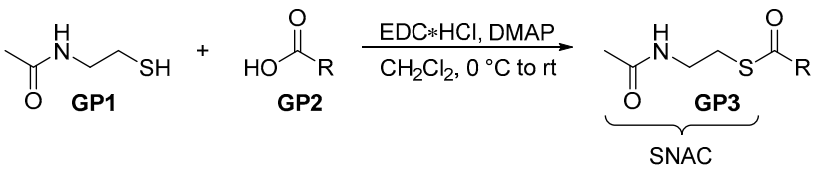
All chemicals and solvents were obtained from Abcr (Karlsruhe, Germany), Acros Organics (Geel, Belgium), BLD Pharm (Kaiserslautern, Germany), Carbolution (St. Ingbert, Germany), Eurisotop (Saarbrücken, Germany), Fluorochem (Hadfield, UK), Grüssing (Filsum, Germany), Roth (Karlsruhe, Germany), Sigma-Aldrich (Schnelldorf, Germany), TCI (Zwijndrecht, Belgium) Thermo Fisher Chemical (Schwerte, Germany), and VWR (Rednor, DE, USA) and were, unless otherwise stated, used without further purification. Dry solvents were obtained from Acros Organics. All reactions were performed under argon gas using dry solvents and reagents. Light-sensitive substances were handled in brown glass- or aluminum-foil-wrapped flasks. The reactions were monitored via TLC using Alugram SilG/UV254 TLC foils from Macherey-Nagel (Düren, Germany). The substances were detected using UV light and a $KMnO_4$ stain (1.50 g of $KMnO_4$, 10.0 g of K_2CO_3, 2.50 mL of 5% NaOH, 200 mL of H_2O). Products were purified via flash chromatography on SiO_2 (Macherey-Nagel MN Kieselgel 60, 40–63 µm). Semi-preparative HPLC was performed using a Waters HPLC (600 controller, 2487 Dual wavelength absorbance detector) and a C18-SP stationary phase (H_2O:MeCN = 95:5 {5 min}, Gradient H_2O:MeCN 95:5 → 5:95 {20 min}, H_2O:MeCN = 5:95 {5 min}, 20 mL/min).

All NMR spectra were recorded using Bruker Avance III HD 500 (Rheinstetten, Germany) with the residual solvent signal as an internal standard: $CDCl_3$ 7.26 ppm for ^1H and 77.16 ppm for ^{13}C.[2] Signal multiplicities are stated, using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad. For ^{13}C-NMR, the following abbreviations were used: q = quarternary, t = tertiary, s = secondary and p = primary. The chemical shifts are reported as values of the δ-scale in [ppm] and the coupling constants *J* in [Hz]. Signal assignments were made with 2D NMR spectra (COSY, HSQC, HMBC, NOESY). High-resolution mass spectra (HRMS) were obtained using a Thermo Fisher Scientific Q Exactive (Orbitrap) mass spectrometer. Optical rotation was recorded on a Jasco P-1020 polarimeter (10 cm cell) from Portman Instruments (Biel-Benken, Switzerland) using the sodium D line (589 nm). The given value of $[\alpha]^D$ represents the average of 50 individual measurements and is stated as $deg \cdot mL \cdot g^{-1} \cdot dm^{-1}$. Elemental analyses were carried out using a 2400 CHN elemental analyzer from Perkin-Elmer (Waltham, MA, USA).

2.2. General Procedures

2.2.1. General Procedure 1: STEGLICH Esterification for SNAC Thioester Formation

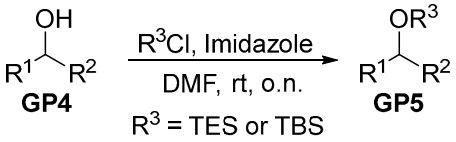
A solution of carboxylic acid (**GP2**, 1.0 eq.) and *N*-acetylcysteamine (**GP1**, 1.5 eq.) in CH_2Cl_2 (0.2 M) was cooled to 0 °C. Subsequently, DMAP (0.1 eq.) and EDC*HCl (1.5 eq.) were added. After warming to room temperature, the solution was stirred for 2 h, before diluting with saturated aqueous NH_4Cl solution. The resulting phases were separated and the aqueous one was extracted three times with CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$ and filtrated. The solvent was removed in vacuo and the crude thioester (**GP3**) was purified via flash chromatography.



Scheme 1. General procedure for thioesterification.

2.2.2. General Procedure 2: Protection of Hydroxyls as Silylethers

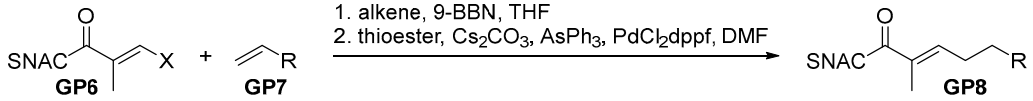
To a stirred solution of secondary alcohol (**GP4**, 1.0 eq.) in DMF (1 M), silylchloride (1.5 eq.) and imidazole (2.5 eq.) were added. After stirring the mixture at room temperature overnight, pentane and water were added. The resulting phases were separated and the aqueous one was extracted three times with pentane. Subsequently, the combined organic phases were washed with brine, dried over $MgSO_4$ and filtrated. The solvent was removed in vacuo and the crude silylether (**GP5**) was purified via filtration over a short plug of silica (pentane).



Scheme 2. General procedure for silylether protection.

2.2.3. General Procedure 3: Hydroboration-SUZUKI-MIYAURI Reaction

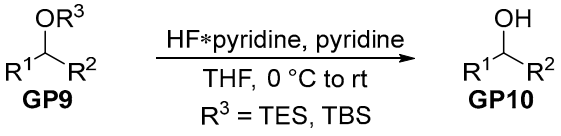
A solution of terminal alkene (**GP7**, 1.5 eq.) in freshly degassed THF (1 M) was cooled to 0 °C and 9-BBN (1.5 eq., 0.5 M in THF) was added dropwise. The mixture was stirred overnight while being allowed to slowly warm to room temperature. Subsequently, freshly degassed DMF (total 0.2 M), thioester vinylhalogenide (**GP6**, 1.0 eq.), $PdCl_2$ (dppf) (5 mol%), $AsPh_3$ (5 mol%) and Cs_2CO_3 (2.0 eq.) were added, and the suspension was heated to 50 °C. After the complete consumption of the starting material (TLC), EtOAc was added, and the mixture was transferred to a separating funnel containing aqueous LiCl solution (10% wt). After the separation and extraction of the aqueous phase using EtOAc (3×), the combined organics were washed with brine and dried over $MgSO_4$. After concentration in vacuo, the crude product (**GP8**) was purified via flash chromatography.



Scheme 3. General procedure for SMR.

2.2.4. General Procedure 4: Deprotection

The silylether (**GP9**, 1.0 eq.) was dissolved in HF-containing stock solution (70% HF*pyridine/pyridine/THF (1:2:8)) at 0 °C. After warming to room temperature and the complete consumption of the starting material according to TLC, the saturated aqueous $NaHCO_3$ solution was added dropwise until no more formation of CO_2 was observed. Subsequently, the phases were separated and the aqueous one was extracted three times using EtOAc. The combined organics were washed with brine and dried over $MgSO_4$. After concentration in vacuo, the crude product (**GP7**) was purified via semi-preparative HPLC.



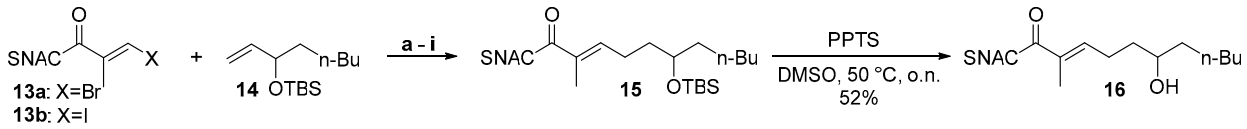
Scheme 4. General procedure for silylether deprotection.

3. Results

Thioester-halides are rare substrates in SMRs. The literature, however, contains an example of the coupling reactions of simple 4-bromothiophenols with 4-tolyl-boronic acid, in which the bulky acyl unit of the thioesters served as a protecting group for the thiol [15]. We used the reported conditions for the synthesis of an ethylenoate that was sensitive to

hydrolysis and a base described by Suzuki et al. as the starting point for our studies [30]. These were carried out using the SNAC (*E*)-3-bromo-2-methylprop-2-enethioate **13a** and the OTBS-protected 3-hydroxyolefin **14** (1.0 equiv. of **13a**, 1.1 equiv. of **14**, 1.1 equiv. 9-BBN, 5mol% $PdCl_2$(dppf) (dppf: 1,1′-bis(diphenylphosphino)ferrocene) and 2.0 equiv. K_2CO_3) (Schemes 1 and 2). Although a basic coupling reactivity was observed, the yields of the reactions varied hardly reproducibly over a wide range and showed a strong dependence on even small variations in the amounts of thioester, alkene, borane and Pd catalyst. This suggests that several side reactions might proceed at rates similar to the desired pathway. We therefore carried out a systematic optimization study (Table 1, see Supplementary Materials pages 3–7).

Table 1. Optimization of the conditions for the coupling of the SNAC thioester halides **13a**/**13b** and TBS-protected olefin **14**.



Entry	X	Base	Additive	Temperature [°C]	Isolated Yield [%]
a	Br	K_2CO_3	-	50	54
b	Br	K_2CO_3	P(*o*-furyl)$_3$	50	23
c	Br	K_2CO_3	$AsPh_3$	50	55
d	Br	Cs_2CO_3	-	50	55
e	Br	K_2CO_3	-	20	13
f	I	K_2CO_3	-	50	55
g	I	Cs_2CO_3	$AsPh_3$	50	34
h	I	Cs_2CO_3	$AsPh_3$	65	-
i	**I**	**Cs_2CO_3**	**$AsPh_3$**	**20**	**78**

General reaction conditions: 1. **14** (1.5 eq., 1 M in THF), 9-BBN (1.5 eq., 0.5 M in THF), 0 °C to 20 °C, o.n.; 2. DMF (total 0.2 M), **13** (1.0 eq.), base (2.0 eq.), $PdCl_2$dppf (5 mol%), additive (5 mol%), reaction control via TLC. Reaction scale: 90–100 µmol.

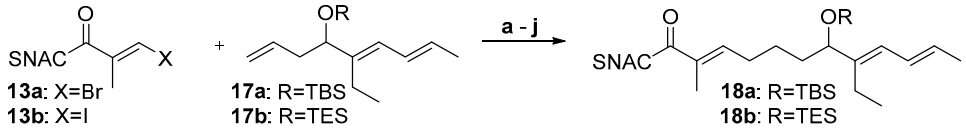
For this, we varied the individual reaction parameters. Since we assumed that the side reactions of the 3-bromoacryl thioate **13a** were a particular problem, we worked with an excess of 1.5 equiv. of alkene **14** and 9-BBN. Different thioester halides (Br and I), bases (K_2CO_3 and Cs_2CO_3), additives (P(*o*-furyl)$_3$ and $AsPh_3$) and temperatures (20 °C, 50 °C and 65 °C) were tested. All reactions were carried out on a scale of 90–100 µmol of **13a/13b** and compared based on the isolated yield after column chromatography. The yields in the basic experiment with an excess of 1.5 equiv. of **14** and 9-BBN (entry a) were fortunately stable upon repetition in a range slightly above 50%. The variation in the individual parameters did not lead to a marked increase in this value, whereas the addition of P(*o*-furyl)$_3$ and the decrease in the reaction temperature even significantly reduced the yield (entries b and e). Fortunately, the combined change in several parameters led to a significantly improved result (entry i). Using 3-iodoacryl thioate **13b**, Cs_2CO_3, $AsPh_3$ and carrying out the reaction at room temperature gave **15** a yield of 78%. The TBS deprotection of **16** achieved a 52% yield using PPTS under conditions that we identified as successful in the synthesis of other SNAC-7-hydroxy-2-enethioates [21–23].

Side products that could not be isolated and fully analyzed were regularly detected in the low-yielding hydroboration SMRs in Table 1. According to TLC, these were highly polar compounds whose migration behavior suggests that they were derived from SNAC. We assume that a major part of this is the homocoupling product of the thioester acrylates **13a/13b** and the 2-(*N*-acetamidyl)-ethylketone resulting after the C–S bond insertion of the Pd, a side reaction described previously for low-functionalized thioesters [15]. The yield improvement observed in the optimization study is consistent with the suppression of these side reactions. Cs_2CO_3 is much more soluble in DMF than K_2CO_3, leading to a much higher

effective concentration of carbonate. This should significantly improve the activation of the *ate* complex for alkyl group transfer to the Pd (step 3 in Figure 2) and accelerate the heterocoupling reaction. The iodoacrylate is more reactive towards Pd insertion than the bromoacrylate, thus favoring this productive reaction (step 1) compared to the insertion of Pd into the C–S bond. This selectivity is expected to be even more pronounced at room temperature than at 50 °C. The addition of $AsPh_3$ supports these effects by accelerating both the formation of the active Pd(0) from the Pd(II) species and transmetallation, due to its lower σ-donor effect than PPh_3 [31,32].

We now turn to the coupling between the thioesters **13a**/**13b** and the olefins **17a** and **17b**, which resemble the sensitive 5-hydroxy-tri-1,3,7-ene in target molecule **12** (Table 2, see SI pages 7–13). Their higher degree of functionalization makes them more susceptible to side reactions during the introduction and removal of the protecting group and during the coupling cascade. In addition to screening the same thioester halides (**13a/13b**) as in Table 2, a broader panel of bases (Cs_2CO_3, K_2CO_3 and K_3PO_4) and hydroxyl protection groups (TBS and TES) on the olefinic coupling partner were examined. Due to the superiority of the previous optimization, only $AsPh_3$ was applied as an additive and only 20 °C and 50 °C were tested as reaction temperatures.

Table 2. Optimization of the conditions for the coupling between **13a**/**13b** and protected trienes **17a**/**17b**, varying protecting group, halogenide, base, additives and temperature.



Entry	X	PG	Base	Additive	Temperature [° C]	Isolated Yield [%]
a	Br	TBS	2 eq. K_2CO_3	-	50	27
b	Br	TBS	3 eq. K_3PO_4	-	50	17
c	Br	TES	2 eq. K_2CO_3	-	50	25
d	Br	TES	3 eq. K_3PO_4	-	50	12
e	Br	TES	2 eq. K_2CO_3	-	20	15
f	I	TES	2 eq. K_2CO_3	-	50	49
g	Br	TES	2 eq. Cs_2CO_3	-	50	80
h	Br	TES	2 eq. K_2CO_3	$AsPh_3$	50	77
i	I	TES	2 eq. K_2CO_3	-	20	63
j	**I**	**TES**	**2 eq. Cs_2CO_3**	**$AsPh_3$**	**20**	**87**

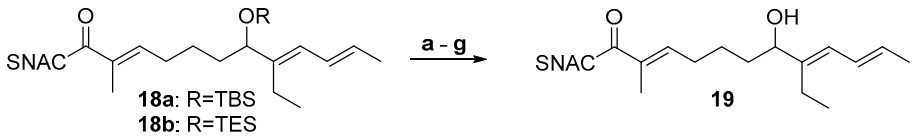
Reaction conditions: 1. **17** (1.5 eq., 1 M in THF), 9-BBN (1.5 eq., 0.5 M in THF), 0 °C to 20 °C, o.n.; 2. DMF (total 0.2 M), **13** (1.0 eq.), base (2.0 eq.), $PdCl_2$dppf (5 mol%), additive (5 mol%), reaction control via TLC.

Compared to the basic experiments (entries a and c, Table 2), a decrease in the yield of **18** was observed when the reaction was carried out at 20 °C instead of 50 °C, or when K_3PO_4 was employed as a base (entries a–e). In contrast to the experiments using the simpler coupling partner **14** (Table 1), the change in one or two reaction parameters led to an increase in the yield of up to 80% (entries f–i). When these measures were combined and the reaction was carried out at 20 °C, a further increase to an 87% yield of **18b** was achieved (entry j, Scheme 3). The TES group was expected to be more easily removable than the TBS group (vide infra). As the former demonstrated stability under the conditions tested, and as both protecting groups gave similar yields in the comparable entries a–d, the optimization in entries f–j was conducted using the TES protection group. The results summarized in Table 2 are in agreement with those observed in Table 1 and confirm the conclusions/interpretations drawn from them.

Numerous side reactions were conceivable during the deprotection of the silyl ethers **18a** and **18b**, such as eliminations, intramolecular oxa-Michael additions or interferences with the thioester. The slightly acidic conditions of the standard deprotection protocol with PPTS (see Table 1, step 2) resulted in the elimination of the alcohol/silylether (entry

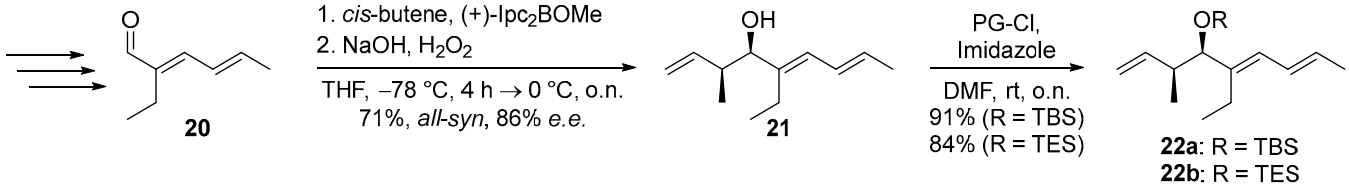
a, Table 3, see SI page 14). With TBAF, the formation of the desired product was also not observed in any case. No reaction of the TBS ether **18a** was found after 1 h at 0 °C (entry b). Decomposition occurred for the TBS ether **18b** after overnight reaction at 20 °C and for the TES ether after only 1 h at 0 °C (entries c and d). Standard HF*pyridine treatment also resulted in decomposition (entry e). A successful procedure was finally adopted from a protocol previously reported by Carreira et al., which relied on using a premixed stock solution of HF*pyridine in THF supplemented with additional pyridine at 0 °C [33]. Deprotection was successful for both silylethers and led to the attainment of the desired alcohol **19** in pure form after column chromatography (entries f and g, Scheme 4). The reactions were continuously monitored by TLC and stopped before noticeable decomposition occurred. The yield for the TES ether was significantly better than that of the TBS ether, suggesting that the former is the preferable protecting group for the synthesis of **12**.

Table 3. Testing conditions for silylether deprotection.



Entry	PG	Reagent	Conditions	Result
a	TBS	PPTS	DMSO, 50 °C, o.n.	Decomposition
b	TBS	TBAF	THF, 0 °C, 1 h	No reaction
c	TBS	TBAF	THF, 0 → 20 °C, o.n.	Decomposition
d	TES	TBAF	THF, 0 °C, 1 h	Decomposition
e	TBS	HF*pyridine	THF, 0 °C, 3 h	Decomposition
f	**TBS**	**HF*pyridine, pyridine**	**THF, 0 → 20 °C, 3 h**	**51%**
g	**TES**	**HF*pyridine, pyridine**	**THF, 0 → 20 °C, 3 h**	**81%**

The reaction sequences to **12** were carried out starting from aldehyde **20** (see Supplementary Materials pages 14–16). Brown crotylation first afforded the highly sensitive hydroxytriene **21** in a yield of 71% and an 86% *e.e.*, which was immediately transformed into the isolatable TBS and TES ethers **22a** and **22b**, with yields of 91% and 84% (Scheme 5). This was followed by the established one-pot two-step cascade of hydroboration and SMR to give **28a** and **28b**, which were deprotected under the optimized conditions to give 7-hydroxy-2-ene-SNAC thioate **12** in overall yields of 16–60% (Table 4, see Supplementary Materials pages 17–19). These results confirm, on the one hand, that TES is the preferable protecting group compared to TBS as it leads to higher yields in both the coupling and deprotection reaction (entries a and b). On the other hand, they show the positive effect of optimizing the SMR conditions, which led to an improvement in the yield from 51% to 74% in the coupling step (entries b and c).

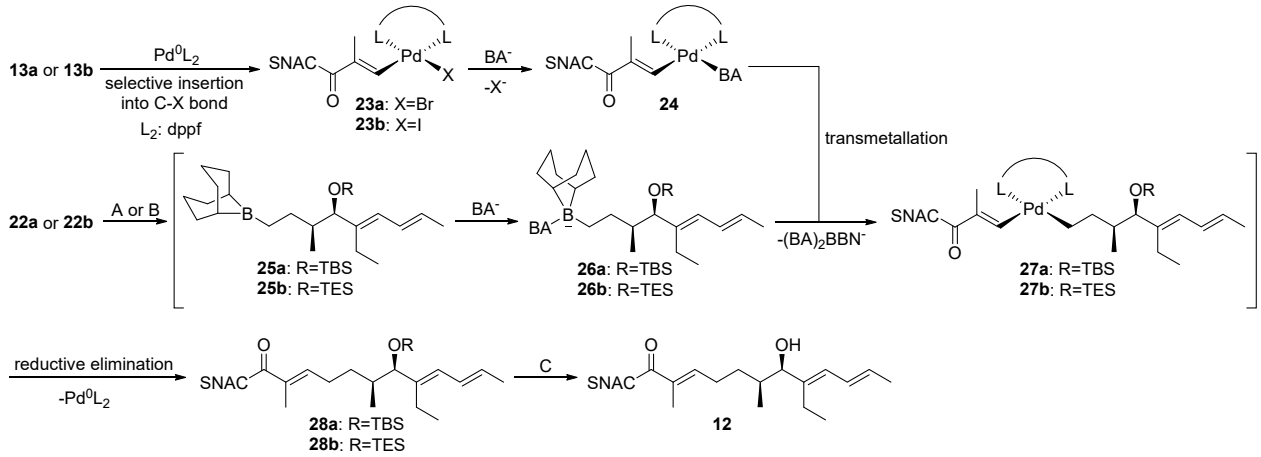


Scheme 5. Synthesis of protected hydroxytrienes **22a**/**22b** from aldehyde **20**. See SI for the steps leading to precursor aldehyde **20**.

To illustrate the broader synthetic utility of the synthesis of chiral 7-hydroxy-2-ene-SNAC thioates, we additionally applied the method to the synthesis of octaketide **33** (Scheme 6, see SI pages 20–24). In comparison to **12**, this compound exhibits a highly hydrophobic heptyl chain, an additional chiral secondary alcohol and a relative *anti*-configuration at the vicinal stereocenters at C-6 and C-7. Olefin **31** was obtained from

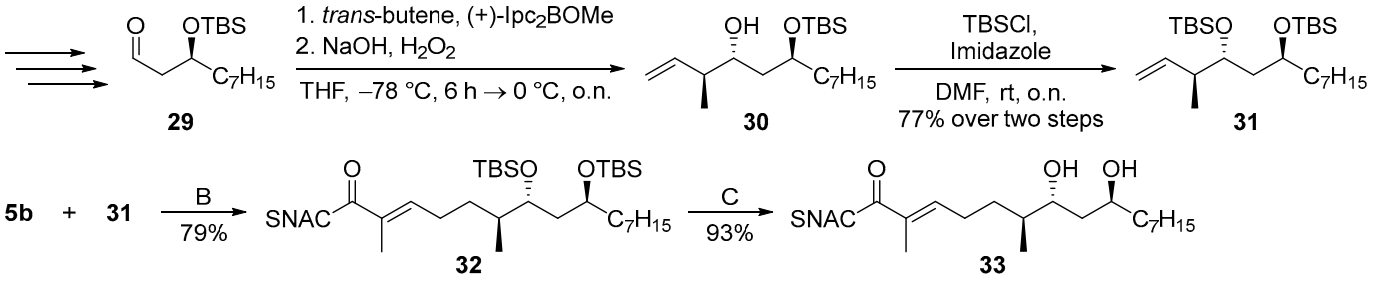
aldehyde **29 via** Brown crotylation and TBS protection. Despite the presence of two sterically demanding TBS groups, the hydroboration to SMR sequence proceeded similarly well, as in the synthesis of **28b**, giving **32** a yield of 79% starting from **31**. The deprotection of **32** was achieved in a 93% yield under the conditions optimized for the synthesis of the sensitive allylic alcohols **12** and **19**. These conditions thus also provide an advantage for the deprotection of SNAC 2-enethioate silyl ethers devoid of critical (poly)enes, as is evident from the comparison made with the deprotection of **15** to **16** that was carried out using PPTS and that led to a yield of only 52%.

Table 4. Two-pot three-step reaction sequence for thioester **12** starting from **13a/13b** and **22a/22b**. The coupling step is formulated under the assumption that the SMR proceeds via a Pd(0)/Pd(II) mechanism.



Entry	X	PG	Conditions	Coupling Yield [%]	Deprotection Yield [%]	Overall Yield [%]
a	Br	TBS	A	30	53	16
b	Br	TES	A	51	86	44
c	**I**	**TES**	**B**	**74**	**81**	**60**

Reaction conditions: A. **22** (1.0 eq., 1 M in THF), 9-BBN (1.0 eq., 0.5 M in THF), 0 °C to 20 °C, o.n.; 2. DMF (total 0.2 M), **13** (1.5 eq.), K_2CO_3 (2.0 eq.), $PdCl_2$dppf (5 mol%), 50 °C, reaction control via TLC; B. **22** (1.5 eq., 1 M in THF), 9-BBN (1.5 eq., 0.5 M in THF), 0 °C to 20 °C, o.n.; 2. DMF (total 0.2 M), **13** (1.0 eq.), Cs_2CO_3 (2.0 eq.), $PdCl_2$dppf (5 mol%), $AsPh_3$ (5 mol%), 20 °C, reaction control via TLC; C. **22** (10.0 mg, 1.0 eq.), 110 µL of HF-containing stock solution (1 part HF*pyridine, 2 parts pyridine, 8 parts THF).



Scheme 6. Synthesis of chiral hydroxythioate **33**. See SI for the steps leading to precursor aldehyde **29**.

4. Discussion

In summary, a useful method for the rapid assembly of SNAC hydroxyenethioates that makes use of a cascade of hydroboration to SMR, followed by optimized silylether deprotection, was developed. The four SNAC hydroxyenethioates **12**, **16**, **19** and **33** were synthesized using this strategy. The chiral **12** and **33** were obtained in four synthetic operations using the aldehydes **20** and **29**, respectively, with overall yields of 36% and 57% and high stereoisomeric purity. This represents a significant improvement over previously described routes to similar compounds, which either required significantly more steps or

gave lower overall yields (eight steps, 10% overall yield for the SNAC thioester analog of **10** starting from propionaldehyde) [21]. Other routes gave SNAC 7-hydroxy-2-ene thioates in five steps from TBS-protected 1,5-hexanediol with a total yield of 23% [20]. The latter, however, only provided access to racemic products, which were also not branched in the α-position. It did also not offer the flexibility in backbone installation that the presented method does.

The SMR-based coupling method presented here is compatible with the presence of SNAC thioesters and can be used in the future for the flexible and efficient preparation of substrate surrogates for studies of IMOMA cyclases and other enzymatic megasynthase domains that act on similar functionalization patterns as those present at C-1–C-6 in **12**, **16**, **19** and **33**. The method should also be of interest for the synthesis of precursors of non-enzymatic IMOMA reactions. It has been shown for chemically catalyzed IMOMA reactions that *cis*-THP stereoselectivity can be more reliably achieved using enethioates rather than enoates, meaning that the former are attractive precursors.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemistry5020096/s1, The Supplementary Materials contain detailed synthetic procedures and analytical data including ^{1}H and ^{13}C NMR spectra in Figures S1–S50. References [34–37] are cited in Supplementary Materials.

Author Contributions: Conceptualization, S.D. and F.H.; investigation, S.D. and L.S.; resources, F.H.; writing—original draft preparation, F.H.; writing—review and editing, S.D., L.S. and F.H.; supervision, F.H.; project administration, F.H.; funding acquisition, F.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Deutsche Forschungsgemeinschaft (DFG), grant numbers HA 5841/5-1 and HA 5841/7-1.

Data Availability Statement: The data presented in this study are available on request from the corresponding author (Frank Hahn).

Acknowledgments: We thank Central Analytics of the Department of Chemistry, as well as the North Bavarian NMR Centre (NBNC) at the University of Bayreuth.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Weissman, K.J.; Müller, R. Protein–Protein Interactions in Multienzyme Megasynthetases. *ChemBioChem* **2008**, *9*, 826–848. [CrossRef]
2. Grininger, M. Enzymology of Assembly Line Synthesis by Modular Polyketide Synthases. *Nat. Chem. Biol.* **2023**, *19*, 401–415. [CrossRef] [PubMed]
3. Ge, H.-M.; Huang, T.; Rudolf, J.D.; Lohman, J.R.; Huang, S.-X.; Guo, X.; Shen, B. Enediyne Polyketide Synthases Stereoselectively Reduce the β-Ketoacyl Intermediates to β-d-Hydroxyacyl Intermediates in Enediyne Core Biosynthesis. *Org. Lett.* **2014**, *16*, 3958–3961. [CrossRef] [PubMed]
4. Sahner, J.H.; Sucipto, H.; Wenzel, S.C.; Groh, M.; Hartmann, R.W.; Müller, R. Advanced Mutasynthesis Studies on the Natural α-Pyrone Antibiotic Myxopyronin from *Myxococcus Fulvus*. *ChemBioChem* **2015**, *16*, 946–953. [CrossRef] [PubMed]
5. Pinto, A.; Wang, M.; Horsman, M.; Boddy, C.N. 6-Deoxyerythronolide B Synthase Thioesterase-Catalyzed Macrocyclization Is Highly Stereoselective. *Org. Lett.* **2012**, *14*, 2278–2281. [CrossRef] [PubMed]
6. Hansen, D.A.; Rath, C.M.; Eisman, E.B.; Narayan, A.R.H.; Kittendorf, J.D.; Mortison, J.D.; Yoon, Y.J.; Sherman, D.H. Biocatalytic Synthesis of Pikromycin, Methymycin, Neomethymycin, Novamethymycin, and Ketomethymycin. *J. Am. Chem. Soc.* **2013**, *135*, 11232–11238. [CrossRef]
7. Franke, J.; Hertweck, C. Biomimetic Thioesters as Probes for Enzymatic Assembly Lines: Synthesis, Applications, and Challenges. *Cell Chem. Biol.* **2016**, *23*, 1179–1192. [CrossRef]
8. Hahn, F.; Kandziora, N.; Friedrich, S.; Leadlay, P.F. Synthesis of Complex Intermediates for the Study of a Dehydratase from Borrelidin Biosynthesis. *Beilstein J. Org. Chem.* **2014**, *10*, 634–640. [CrossRef]
9. Berkhan, G.; Merten, C.; Holec, C.; Hahn, F. The Interplay between a Multifunctional Dehydratase Domain and a C-Methyltransferase Effects Olefin Shift in Ambruticin Biosynthesis. *Angew. Chem. Int. Ed.* **2016**, *55*, 13589–13592. [CrossRef]

10. Schröder, M.; Roß, T.; Hemmerling, F.; Hahn, F. Studying a Bottleneck of Multimodular Polyketide Synthase Processing: The Polyketide Structure-Dependent Performance of Ketoreductase Domains. *ACS Chem. Biol.* **2022**, *17*, 1030–1037. [CrossRef]
11. Wunderlich, J.; Roß, T.; Schröder, M.; Hahn, F. Step-Economic Synthesis of Biomimetic β-Ketopolyene Thioesters and Demonstration of Their Usefulness in Enzymatic Biosynthesis Studies. *Org. Lett.* **2020**, *22*, 4955–4959. [CrossRef]
12. Sundaram, S.; Kim, H.J.; Bauer, R.; Thongkongkaew, T.; Heine, D.; Hertweck, C. On-Line Polyketide Cyclization into Diverse Medium-Sized Lactones by a Specialized Ketosynthase Domain. *Angew. Chem. Int. Ed.* **2018**, *57*, 11223–11227. [CrossRef]
13. Hooshmand, S.E.; Heidari, B.; Sedghi, R.; Varma, R.S. Recent Advances in the Suzuki–Miyaura Cross-Coupling Reaction Using Efficient Catalysts in Eco-Friendly Media. *Green Chem.* **2019**, *21*, 381–405. [CrossRef]
14. Miyaura, N.; Suzuki, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chem. Rev.* **1995**, *95*, 2457–2483. [CrossRef]
15. Zeysing, B.; Gosch, C.; Terfort, A. Protecting Groups for Thiols Suitable for Suzuki Conditions. *Org. Lett.* **2000**, *2*, 1843–1845. [CrossRef]
16. Liebeskind, L.S.; Srogl, J. Thiol Ester−Boronic Acid Coupling. A Mechanistically Unprecedented and General Ketone Synthesis. *J. Am. Chem. Soc.* **2000**, *122*, 11260–11261. [CrossRef]
17. Cheng, H.-G.; Chen, H.; Liu, Y.; Zhou, Q. The Liebeskind–Srogl Cross-Coupling Reaction and Its Synthetic Applications. *Asian J. Org. Chem.* **2018**, *7*, 490–508. [CrossRef]
18. Meng, S.; Tang, G.-L.; Pan, H.-X. Enzymatic Formation of Oxygen-Containing Heterocycles in Natural Product Biosynthesis. *ChemBioChem* **2018**, *19*, 2002–2022. [CrossRef]
19. Hemmerling, F.; Hahn, F. Biosynthesis of Oxygen and Nitrogen-Containing Heterocycles in Polyketides. *Beilstein J. Org. Chem.* **2016**, *12*, 1512–1550. [CrossRef] [PubMed]
20. Pöplau, P.; Frank, S.; Morinaka, B.I.; Piel, J. An Enzymatic Domain for the Formation of Cyclic Ethers in Complex Polyketides. *Angew. Chem. Int. Ed.* **2013**, *52*, 13215–13218. [CrossRef]
21. Berkhan, G.; Hahn, F. A Dehydratase Domain in Ambruticin Biosynthesis Displays Additional Activity as a Pyran-Forming Cyclase. *Angew. Chem. Int. Ed.* **2014**, *53*, 14240–14244. [CrossRef] [PubMed]
22. Hollmann, T.; Berkhan, G.; Wagner, L.; Sung, K.H.; Kolb, S.; Geise, H.; Hahn, F. Biocatalysts from Biosynthetic Pathways: Enabling Stereoselective, Enzymatic Cycloether Formation on a Gram Scale. *ACS Catal.* **2020**, *10*, 4973–4982. [CrossRef]
23. Wagner, L.; Stang, J.; Derra, S.; Hollmann, T.; Hahn, F. Towards Understanding Oxygen Heterocycle-Forming Biocatalysts: A Selectivity Study of the Pyran Synthase PedPS7. *Org. Biomol. Chem.* **2022**, *20*, 9645–9649. [CrossRef]
24. Sung, K.H.; Berkhan, G.; Hollmann, T.; Wagner, L.; Blankenfeldt, W.; Hahn, F. Insights into the Dual Activity of a Bifunctional Dehydratase-Cyclase Domain. *Angew. Chem. Int. Ed.* **2018**, *57*, 343–347. [CrossRef]
25. Wagner, L.; Roß, T.; Hollmann, T.; Hahn, F. Cross-Linking of a Polyketide Synthase Domain Leads to a Recyclable Biocatalyst for Chiral Oxygen Heterocycle Synthesis. *RSC Adv.* **2021**, *11*, 20248–20251. [CrossRef]
26. Wagner, D.T.; Zhang, Z.; Meoded, R.A.; Cepeda, A.J.; Piel, J.; Keatinge-Clay, A.T. Structural and Functional Studies of a Pyran Synthase Domain from a Trans-Acyltransferase Assembly Line. *ACS Chem. Biol.* **2018**, *13*, 975–983. [CrossRef] [PubMed]
27. Ueoka, R.; Uria, A.R.; Reiter, S.; Mori, T.; Karbaum, P.; Peters, E.E.; Helfrich, E.J.N.; Morinaka, B.I.; Gugger, M.; Takeyama, H.; et al. Metabolic and Evolutionary Origin of Actin-Binding Polyketides from Diverse Organisms. *Nat. Chem. Biol.* **2015**, *11*, 705–712. [CrossRef]
28. Luhavaya, H.; Dias, M.V.B.; Williams, S.R.; Hong, H.; de Oliveira, L.G.; Leadlay, P.F. Enzymology of Pyran Ring A Formation in Salinomycin Biosynthesis. *Angew. Chem. Int. Ed.* **2015**, *54*, 13622–13625. [CrossRef]
29. Woo, A.J.; Strohl, W.R.; Priestley, N.D. Nonactin Biosynthesis: The Product of NonS Catalyzes the Formation of the Furan Ring of Nonactic Acid. *Antimicrob. Agents Chemother.* **1999**, *43*, 1662–1668. [CrossRef] [PubMed]
30. Miyaura, N.; Ishiyama, T.; Sasaki, H.; Ishikawa, M.; Sato, M.; Suzuki, A. Palladium-Catalyzed Inter- and Intramolecular Cross-Coupling Reactions of B-Alkyl-9-Borabicyclo [3.3.1] Nonane Derivatives with 1-Halo-1-Alkenes or Haloarenes. Syntheses of Functionalized Alkenes, Arenes, and Cycloalkenes via a Hydroboration-Coupling Sequence. *J. Am. Chem. Soc.* **1989**, *111*, 314–321. [CrossRef]
31. Farina, V.; Krishnan, B. Large Rate Accelerations in the Stille Reaction with Tri-2-Furylphosphine and Triphenylarsine as Palladium Ligands: Mechanistic and Synthetic Implications. *J. Am. Chem. Soc.* **1991**, *113*, 9585–9595. [CrossRef]
32. Chishiro, A.; Konishi, M.; Inaba, R.; Yumura, T.; Imoto, H.; Naka, K. Tertiary Arsine Ligands for the Stille Coupling Reaction. *Dalton Trans.* **2021**, *51*, 95–103. [CrossRef] [PubMed]
33. Carreira, E.M.; Du Bois, J. (+)-Zaragozic Acid C: Synthesis and Related Studies. *J. Am. Chem. Soc.* **1995**, *117*, 8106–8125. [CrossRef]
34. Li, Y.; Zhang, W.; Zhang, H.; Tian, W.; Wu, L.; Wang, S.; Zheng, M.; Zhang, J.; Sun, C.; Deng, Z.; et al. Structural Basis of a Broadly Selective Acyltransferase from the Polyketide Synthase of Splenocin. *Angew. Chem.* **2018**, *130*, 5925–5929. [CrossRef]
35. Campbell, N.E.; Sammis, G.M. Single-Electron/Pericyclic Cascade for the Synthesis of Dienes. *Angew. Chem. Int. Ed.* **2014**, *53*, 6228–6231. [CrossRef]

36. Whittaker, A.M.; Lalic, G. Monophasic Catalytic System for the Selective Semireduction of Alkynes. *Org. Lett.* **2013**, *15*, 1112–1115. [CrossRef]
37. Baldwin, J.E.; Moloney, M.; Parsons, A.F. An intramolecular cobalt cyclisation for the construction of substituted pyrrolidines. *Tetrahedron* **1992**, *48*, 9373–9384. [CrossRef]