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Natural products in parallel synthesis: Triazole libraries of nonactic acid

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ABSTRACT

The synthesis of a library of nonactic acid-derived triazoloamide derivatives and their evaluation as antimicrobial agents is described.

The development of pharmaceutical agents often relies on compounds prepared from natural products or structures inspired by natural products.[1,2] We have been interested in straightforward synthetic methods that combine the stereochemical and topological complexity of natural product scaffolds with the diverse analogue generation available from combinatorial synthesis to produce diverse, pharmacologically interesting compounds.

Nonactin[3] (**1**) is a macrotetrolide, antibiotic, natural product produced by *Streptomyces griseus*. The macrocyclic ring of nonactin is composed of two units of (+)-nonactate and two units of (−)-nonactate in an alternating arrangement of (+)-(−)-(+)-(−), such that nonactin is achiral.[4–9]

Methanolysis of nonactin followed by resolution using *Rhodococcus* under aerobic and anaerobic conditions provides both enantiomers of methyl nonactate (Fig. 1).[10] We have been interested in the monomeric (+) and (−)-methyl nonactate (**2**) units as complex natural product scaffolds for combinatorial compound libraries. Incorporation of the nonactic acid building block in diversity-oriented synthesis can efficiently generate a highly diverse library with relatively complex stereochemistry. Given the biological activity of the precursor, we hypothesized that synthetic derivatives of methyl nonactate would be an excellent place to identify new molecules with interesting pharmacological activity. We proposed to examine these compounds in a series of antimicrobial assays to test this hypothesis.

1,2,3-Triazoles are a significant class of heterocyclic compounds, generally prepared from a 1,3-dipolar addition of terminal alkynes and azides. Recent reports have described the synthesis and testing of series of 1,2,3-triazoles,[11–15] and significant biological activities have been observed for several triazole ring-based

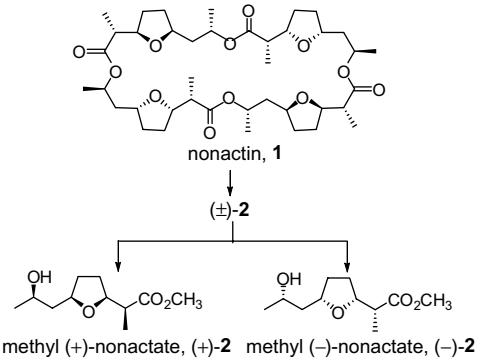
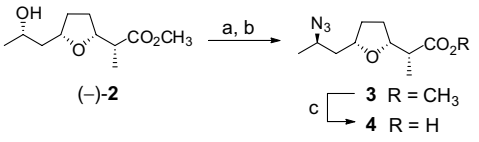


Figure 1. Nonactin and the key starting component methyl nonactate.



Scheme 1. Reagents and conditions: (a) *p*-toluenesulfonyl chloride, pyridine; (b) NaN_3, DMF, 50 °C, 66% (2 steps); (c) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O (2:1:1), 95%.

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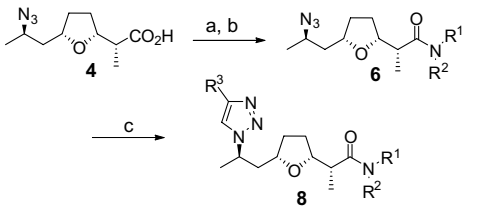

Scheme 2. Reagents and conditions: (a) oxalyl chloride, benzene, 50 °C; (b) **5**, poly(vinylpyridine), CH_2Cl_2, **6**{*1*}, 54%; **6**{*2*}, 66%; **6**{*3*}, 79%; **6**{*4*}, 73%; **6**{*5*}, 53%; (c) **7**, $CuSO_4$, sodium ascorbate, MW, 100 °C, 5 min, 27–97%.

derivatives. Our goal was to integrate the nonactic acid scaffold with the potential pharmacophore of a substituted triazole ring.

The copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition is an efficient one-pot method to access triazoles.[15–17] Given our interest in triazole-substituted nonactic acid, azide-substituted nonactic acid (**4**) was targeted as the starting scaffold for nonactic acid-based libraries. Azide **4** has previously been synthesized from methyl nonactate via a double inversion procedure through a bromide intermediate.[18]

Our straightforward synthesis of azido acid **4** from hydroxyester **2** is shown in Scheme 1. (−)-Methyl nonactate ((−)-**2**) was converted to the tosylate ester followed by substitution with sodium azide (DMF, 50 °C, 5 h) to provide azidoester **3**. Ester hydrolysis provided azido-nonactic acid (**4**) in excellent yield.

As shown in Scheme 2, synthesis of a library of triazoloamides began with conversion of nonactic acid **4** to the corresponding amide **6** through formation of the acid chloride, followed by reaction with amines **5** and poly(vinylpyridine). The triazoloamide **8** was produced through a microwave assisted copper-catalyzed 1,3-dipolar addition of amide **6** with terminal alkyne **7**.

Five amine building blocks (Fig. 2) were used to produce a series of amides. One primary amine (**5**{*5*}) and four secondary amines were selected.

To produce the 161-member triazoloamide library, 32 terminal alkynes (**7**{*1–35*}) were selected (Fig. 3). While many terminal alkynes are commercially available, several alkynes were independently synthesized to increase the diversity of substituted triazoloamide products. Propargyl alcohol or amine and the requisite chloroformate were used to prepare alkynes **7**{*20*} and **7**{*27*}. The necessary isocyanate was reacted with propargyl alcohol or amine to prepare alkynes **7**{*28–30*}. Alkynes **7**{*19*} and **7**{*31–35*} were prepared from propargyl bromide and substituted phenols or amines.

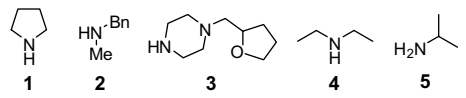

Figure 2. Amine building blocks **5**{*1–5*} for the synthesis of amide **6**.

The general triazole synthesis was modified from previously published one-pot procedures[17,19,20] and optimized for our system. The best general conditions were found to be 100 mol% alkyne, 100 mol% $CuSO_4$, and 33 mol% sodium ascorbate, relative to azidoamide **6**, in *tert*-butanol/H_2O (1:1) submitted to microwave irradiation at 100 °C for 5 min.[21] All compounds were obtained by a simple aqueous work-up followed by silica plug purification.

While a lower copper catalyst loading did provide the desired product, a full equivalent was most efficient for driving all variations of alkyne building blocks to completion. Higher levels of $CuSO_4$ were most significant for reaction completion in the cases of nitrogen-containing alkynes, specifically **7**{*23–30*}.

Table 1 presents the results for the synthesis of 161 triazoloamide library compounds (27-97%). Library members are identified using the 'Chemset' brace numbering system. Compound **8**{*2,4*} refers to use of amine **5**{*2*} and alkyne**7**{*4*}. For example, *N*-benzylmethylamine (Fig. 2, compound **5**{*2*}) is used to produce the corresponding amide **6**{*2*}. Reaction of amide **6**{*2*} with 4-ethynylbiphenyl (Fig. 3, **7**{*4*}) provides triazoloamide **8**{*2,4*}.

All library members were analyzed by LC/MS and one-fifth of the isolated products were structurally verified by ^{1}H NMR analysis. In all cases, a single regioisomeric product was observed by ^{1}H NMR corresponding to the 1,4-disubstituted addition product, as expected for the Cu-catalyzed reaction.[15,17]

Three alkynes (**7**{*9*}, **7**{*13*}, **7**{*14*}) were observed to consistently result in low conversion to triazoloamide and recovery of significant azide starting material (**6**). While there is no clear explanation for the failure of the reactions using these alkynes, solubility problems were encountered using standard reaction conditions.

With a set of nonactic acid derivatives in hand, we examined their activity against a small panel of Gram-positive and Gram-negative bacteria as well as yeast/fungi (Table 2). All compounds were initially screened at a single concentration (1 mM) using the Alamar Blue dye reduction assay. Those compounds showing more than marginal activity were then assayed at a range of dilutions to measure the minimal inhibitory concentration (MIC; lowest concentration at which no dye reduction is observed).

Fourteen compounds (9%) showed weak to moderate activity against one or more microorganisms. In general, most of the antimicrobial activity was focused on the Gram-positive bacteria and

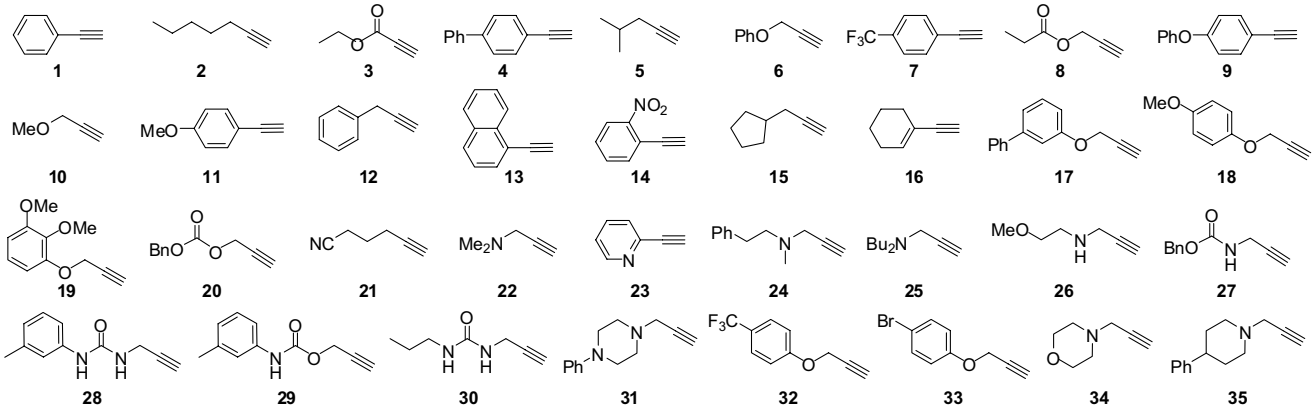

Figure 3. Alkyne building blocks (**7**{*1–35*}) used in triazoloamide library synthesis.

Table 1
Nonactic acid-derived triazoloamide library[21]

Compound	Yield[a] (%)	Compound	Yield[a] (%)	Compound	Yield[a] (%)	Compound	Yield[a] (%)	Compound	Yield[a] (%)
8{*1,1*}	58	**8**{*2,1*}	50	**8**{*3,1*}	62	**8**{*4,1*}	68	**8**{*5,1*}	60
8{*1,2*}	36	**8**{*2,2*}	60	**8**{*3,2*}	38	**8**{*4,2*}	72	**8**{*5,2*}	62
8{*1,3*}	43	**8**{*2,3*}	90	**8**{*3,3*}	36	**8**{*4,3*}	64	**8**{*5,3*}	53
8{*1,4*}	72	**8**{*2,4*}	86	**8**{*3,4*}	57	**8**{*4,4*}	57	**8**{*5,4*}	54
8{*1,5*}	53	**8**{*2,5*}	97	**8**{*3,5*}	34	**8**{*4,5*}	59	**8**{*5,5*}	57
8{*1,6*}	96	**8**{*2,6*}	84	**8**{*3,6*}	30	**8**{*4,6*}	63	**8**{*5,6*}	60
8{*1,7*}	72	**8**{*2,7*}	90	**8**{*3,7*}	52	**8**{*4,7*}	58	**8**{*5,7*}	66
8{*1,8*}	63	**8**{*2,8*}	48	**8**{*3,8*}	38	**8**{*4,8*}	66	**8**{*5,8*}	33
8{*1,9*}	—	**8**{*2,9*}	—	**8**{*3,9*}	—	**8**{*4,9*}	—	**8**{*5,9*}	—
8{*1,10*}	48	**8**{*2,10*}	48	**8**{*3,10*}	45	**8**{*4,10*}	62	**8**{*5,10*}	67
8{*1,11*}	59	**8**{*2,11*}	82	**8**{*3,11*}	48	**8**{*4,11*}	72	**8**{*5,11*}	62
8{*1,12*}	57	**8**{*2,12*}	51	**8**{*3,12*}	28	**8**{*4,12*}	55	**8**{*5,12*}	63
8{*1,13*}	—	**8**{*2,13*}	—	**8**{*3,13*}	—	**8**{*4,13*}	—	**8**{*5,13*}	—
8{*1,14*}	—	**8**{*2,14*}	—	**8**{*3,14*}	68	**8**{*4,14*}	—	**8**{*5,14*}	—
8{*1,15*}	70	**8**{*2,15*}	65	**8**{*3,15*}	44	**8**{*4,15*}	61	**8**{*5,15*}	52
8{*1,16*}	50	**8**{*2,16*}	55	**8**{*3,16*}	51	**8**{*4,16*}	72	**8**{*5,16*}	59
8{*1,17*}	69	**8**{*2,17*}	89	**8**{*3,17*}	53	**8**{*4,17*}	36	**8**{*5,17*}	62
8{*1,18*}	78	**8**{*2,18*}	89	**8**{*3,18*}	44	**8**{*4,18*}	52	**8**{*5,18*}	60
8{*1,19*}	58	**8**{*2,19*}	87	**8**{*3,19*}	43	**8**{*4,19*}	61	**8**{*5,19*}	53
8{*1,20*}	45	**8**{*2,20*}	41	**8**{*3,20*}	27	**8**{*4,20*}	63	**8**{*5,20*}	57
8{*1,21*}	83	**8**{*2,21*}	34	**8**{*3,21*}	51	**8**{*4,21*}	60	**8**{*5,21*}	65
8{*1,22*}	72	**8**{*2,22*}	97	**8**{*3,22*}	43	**8**{*4,22*}	60	**8**{*5,22*}	60
8{*1,23*}	69	**8**{*2,23*}	56	**8**{*3,23*}	50	**8**{*4,23*}	69	**8**{*5,23*}	55
8{*1,24*}	56	**8**{*2,24*}	90	**8**{*3,24*}	94	**8**{*4,24*}	40	**8**{*5,24*}	51
8{*1,25*}	73	**8**{*2,25*}	85	**8**{*3,25*}	53	**8**{*4,25*}	67	**8**{*5,25*}	45
8{*1,26*}	72	**8**{*2,26*}	62	**8**{*3,26*}	35	**8**{*4,26*}	64	**8**{*5,26*}	56
8{*1,27*}	57	**8**{*2,27*}	45	**8**{*3,27*}	60	**8**{*4,27*}	54	**8**{*5,27*}	57
8{*1,28*}	64	**8**{*2,28*}	100[b]	**8**{*3,28*}	42	**8**{*4,28*}	92	**8**{*5,28*}	71
8{*1,29*}	73	**8**{*2,29*}	49	**8**{*3,29*}	76	**8**{*4,29*}	78	**8**{*5,29*}	55
8{*1,30*}	82	**8**{*2,30*}	77	**8**{*3,30*}	58	**8**{*4,30*}	76	**8**{*5,30*}	77
8{*1,31*}	100[b]	**8**{*2,31*}	76	**8**{*3,31*}	35	**8**{*4,31*}	55	**8**{*5,31*}	67
8{*1,32*}	73	**8**{*2,32*}	61	**8**{*3,32*}	53	**8**{*4,32*}	63	**8**{*5,32*}	63
8{*1,33*}	58	**8**{*2,33*}	56	**8**{*3,33*}	49	**8**{*4,33*}	76	**8**{*5,33*}	68
8{*1,34*}	71	**8**{*2,34*}	61	**8**{*3,34*}	43	**8**{*4,34*}	73	**8**{*5,34*}	73
8{*1,35*}	78	**8**{*2,35*}	62	**8**{*3,35*}	68	**8**{*4,35*}	68	**8**{*5,35*}	68

[a] Compounds were determined to be >90% pure by ^{1}H NMR or LC/MS analysis.
[b] Compounds exhibited a purity level of >80% by ^{1}H NMR or LC/MS.

Table 2
Minimum inhibitory concentrations[a,b] (μg/mL)

Compound	Gram-positive bacteria				Gram-negative bacteria		Yeast/fungi		
	Bacillus anthracis	*Bacillus cereus*	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Cryptococcus neoformans*	*Saccharomyces cerevisiae*
8{*2,2*}	430	640						430	430
8{*2,15*}	440	440						219	220
8{*2,23*}	>430[c]								
8{*2,24*}	250	250	250	500				500	
8{*2,25*}	500	250	250	500				250	
8{*3,3*}	>480[c]	240	240	120	120	480	240	480	240
8{*3,4*}			560						
8{*3,7*}			820						
8{*3,13*}	263	260	790						
8{*3,17*}	>590[c]	150	880				590	290	290
8{*3,25*}			820						
8{*3,29*}		570							
8{*4,19*}								480	
8{*4,28*}			700						

[a] Measured by Alamar Blue dye reduction assay. A blank indicates that no inhibition was observed at the maximum test concentration of 1 mM.
[b] Only compounds from the total set of 161 that showed activity in at least one assay are included in the table. Compounds not listed were found to be inactive in all assays.
[c] '>' shows that some inhibition was seen at this concentration although at this highest test concentration, some growth was observed and so a MIC value could not be reliably estimated.

yeast or fungi. The only compound which showed activity against Gram-negative bacteria was **8**{*3,3*}, which showed a very broad spectrum of activity with MIC values of 120 μg/mL against *Staphylococcus aureus* and *Escherichia coli*.

In summary, we have shown that the nonactic acid building block can be readily incorporated into a parallel synthetic scheme. This provides a diverse library with relatively complex stereochemistry. More importantly, this compound library has been used to identify novel antimicrobial leads.

Acknowledgments

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21. *General experimental.* Azidoamide **6** (1.0 equiv) and alkyne **7** (1.0 equiv) were combined in a 2.0 mL microwave vial and suspended in *t*-BuOH/H_2O (1:1, 0.6 mL). $CuSO_4{\cdot}5H_2O$ (1.0 M in H_2O, 1.0 equiv) and sodium ascorbate (0.5 M in H_2O, 0.33 equiv) were added. The reaction was submitted to microwave irradiation (100 °C for 5 min). 1.0 mL 50% NH_4OH was added, followed by 1.0 mL CH_2Cl_2. Organic extracts were collected using a Biotage phase separator, washed with H_2O, and collected through the phase separator. Compounds were purified via a silica plug. A forerun of hexanes removed nonpolar impurities; desired products were eluted with 10% MeOH in CH_2Cl_2 to provide **8**.