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Anti-MRSA Agent Discovery Using Diversity-Oriented Synthesis**

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Antibacterial drugs have played an essential role in the global increase in quality of life and life expectancy. However, these gains are at serious risk owing to bacterial drug resistance by so-called "superbugs", such as methicillin-resistant *Staphylococcus aureus* (MRSA).[1,2] The discovery of new antibiotics with novel modes of action is vital to tackle the threat of multidrug-resistant bacteria. Traditionally, antibiotics have been discovered from natural sources;[3–7] however, there are many disadvantages to using extracts (e.g. limited availability, bioactive constituent identification, and complex analogue synthesis). These problems have led to a complementary approach of synthesizing structurally diverse, natural-product-like small molecules directly and efficiently,[8] an approach known as diversity-oriented synthesis (DOS).[9–15] Whereas compound collections of a common scaffold decorated with diverse building blocks have been synthesized efficiently,[16] there are limited examples of the synthesis of small molecules with a high degree of skeletal diversity (usually by a build–couple–pair strategy).[17–24] Previously, we have used a diazoacetate starting unit to mimic nature's divergent synthetic strategy with acetyl CoA (by a pluripotent functional-group strategy) to synthesize compounds with natural-product scaffolds (e.g. cocaine and warfarin).[24] Herein, we report the use of a solid-supported phosphonate unit to synthesize 242 drug-like compounds based on 18 natural-product-like scaffolds in two to five steps and their use in discovering a new structural class of antibiotic with anti-MRSA activity.

The solid-supported phosphonate **1** (Scheme 1) was identified as an attractive DOS starting unit for three key reasons. First, the reactive phosphonate functionality permits the stereoselective formation of α,β-unsaturated acyl imidazolidinones (**2**) that could be used to generate enantioselectively a wide range of scaffolds that can be diversified further. Second, the imidazolidinone linker not only enables two-point binding of chiral catalysts but also permits divergent cleavage of the exocyclic acyl group (hydrolysis, reduction, esterification, and amide formation). Thirdly, immobilization of **1** on a silyl polystyrene support[25] simplified reaction optimization and work-up procedures in the multistep parallel synthesis (total of over 1000 individual steps), thereby allowing the efficient production of milligram quantities of 242 compounds without the requirement for automation equipment.

In the first step of the diversity-oriented synthesis, **1** was treated with aldehyde building blocks (aryl, heteroaryl, and alkyl; see the Supporting Information) to deliver twelve α,β-unsaturated acyl imidazolidinones (**2**).[26] The second steps of the solid-supported synthesis exploited three catalytic, enantioselective, divergent reaction pathways (Scheme 1): 1) [2+3] cycloaddition (reaction b, *ee* 60–65%, *de* 78–99%),[27] 2) dihydroxylation (reaction c, *ee* 88–91%),[28] and 3) [4+2] cycloaddition (reaction d, *ee* 89–98%, *de* 74–74%).[29] Similar selectivities were observed when repeating the reactions in solution with a triisopropylsilyl-protected linker (as opposed to the diisopropylpolystyrene group; see the Supporting Information). The reactions were also conducted with achiral catalysts to give racemic products, which were used for the later steps of the synthesis. This procedure enabled the diversity-oriented synthesis to be streamlined to half the size, yet permitted the enantioselective synthesis of hits during the structure–activity relationship stages of this

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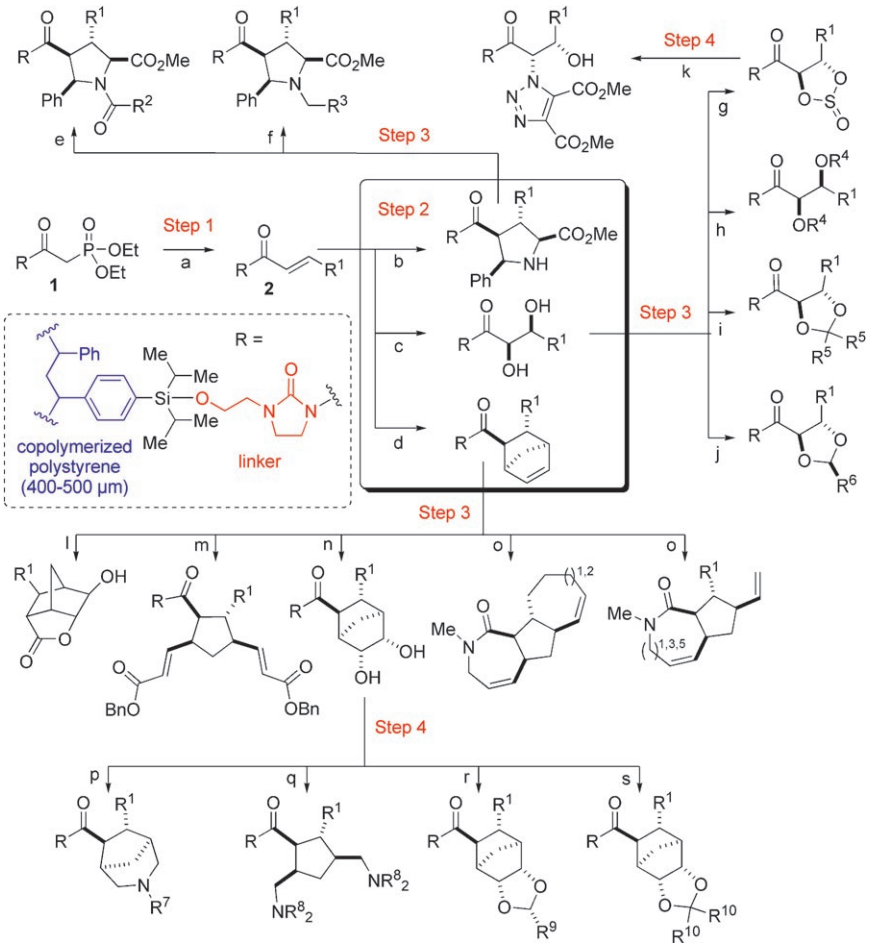
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Diversity-oriented synthesis of 242 compounds based on 18 discrete molecular frameworks. Reagents and conditions: a) LiBr, 1,8-diazabicyclo[5.4.0]undec-7-ene, R^1CHO, MeCN; b) (*R*)-QUINAP, AgOAc, *i*Pr_2NEt, THF, −78 °C→25 °C; c) AD-mix, (DHQD)PHAL, THF/H_2O (1:1); d) chiral bis(oxazoline), $Cu(OTf)_2$, 3 Å M.S., CH_2Cl_2, C_5H_6; e) R^2COCl, DMAP, pyridine, CH_2Cl_2; f) R^3CHO, BH_3·pyridine, MeOH; g) $SOCl_2$, pyridine, CH_2Cl_2, 40 °C; h) R^4Br, Ag_2O, CH_2Cl_2, 40 °C; i) $R^5C(O)R^5$, TsOH, DMF, 65 °C; j) R^6CHO, TsOH, DMF, 65 °C; k) NaN_3, DMF, 100 °C then dimethyl acetylenedicarboxylate, PhMe, 65 °C; l) *m*CPBA, CH_2Cl_2 then MeOH, 65 °C; m) CH_2=$CHCO_2Bn$, PhMe, 120 °C, Grubbs II, CH_2=CH_2; n) OsO_4, NMO, $CH_3C(O)CH_3$/H_2O (10:1); o) RNH_2, Me_2AlCl, PhMe 120 °C; then NaH, $R^{11}X$, DMF, THF; then PhMe, 120 °C, Grubbs II, CH_2=CH_2; p) $NaIO_4$, THF/H_2O (1:1); then R^7NH_2, $NaB(OAc)_3H$, CH_2Cl_2; q) $NaIO_4$, THF/H_2O (1:1); then R^8NHR^8, $NaB(OAc)_3H$, CH_2Cl_2; r) R^9CHO, DMF, TsOH, 60 °C; s) $R^{10}C(O)R^{10}$, DMF, TsOH, 60 °C. DMF = *N*,*N*-dimethylformamide, THF = tetrahydrofuran, DMAP = *N*,*N*-dimethylaminopyridine, (DHQD)PHAL = hydroquinidine 1,4-phthalazinediyl diether, *m*CPBA = *meta*-chloroperbenzoic acid, Ts = *para*-toluenesulfonyl, Grubbs II = 1,3-(bis-(mesityl)-2-imidazolidinyl-idene) dichloro (phenylmethylene) (tricyclohexylphosphine) ruthenium, NMO = 4-methylmorpholine-*N*-oxide, OTf = CF_3SO_3, Bn = benzyl, QUINAP = 1-(2-diphenylphosphino-1-naphthyl)isoquinoline.

work. Later steps involved complexity-generating reactions to diversify the molecular frameworks further and to release divergently the compounds from the solid support. For example, reaction b (step 2) involved the enantioselective 1,3-dipolar cycloaddition of **2** with a wide range of azomethine ylides complexed to Ag^+ and (*R*)- or (*S*)-QUINAP and subsequent acylation or alkylation of the resultant pyrrolidine (reactions e and f, respectively),[27] and cleavage (step 4).

Norbornenes could be synthesized using Evans's asymmetric Diels–Alder methodology (reaction d)[29] and used in divergent reactions l–o. Two highlights in the diversity-oriented synthesis are 1) the tandem ring closing–opening–closing metathesis reaction[30] (reaction o, step 3) to give skeletally diverse tricyclic products (7-5-7, 7-5-8) and 2) the formation of *cis*-fused [3.2.1] bicyclic amines by oxidative cleavage and tandem reductive amination with primary amines (reaction p, step 4). In order to discover a new antibiotic with a novel mode of action, the diversity-oriented synthesis was designed to populate new areas of chemical space so that several of the scaffolds generated are either rare or have no known representation in nature (e.g. the *cis*–*trans*-fused 7-5-7 tricycle resulting from reaction o). Using the chemistry shown in Scheme 1 and a limited number of structurally diverse building blocks, the diversity-oriented synthesis was achieved of 242 small molecules that have 18 molecular frameworks among other unique structural features. The library was made using parallel synthetic techniques leading to 1–20 mg of each final product (molecular-weight range 153–857, mean value 379 g mol^{-1}). All library members were assessed for their identity and quality and purified if necessary by recrystallization, chromatography, or extraction to ensure greater than 90% purity of final products (as determined by ^{1}H NMR spectroscopy, HPLC, and LCMS). Full characterization of the majority (63%) of the final compounds was undertaken; ^{1}H NMR spectroscopy and LCMS characterized the rest.

To assess the degree of overall diversity obtained in this diversity-oriented synthesis, we compared[24] the structural diversity of our library to the chemical space spanned by "benchmark collections": 1) known pharmacologically active small molecules (MDL Drug Data Report database with a molecular-weight cutoff of 650 g mol^{-1} to compare size-independent diversity),[31] 2) 3762 compounds marked as "antibacterials" in the MDDR database,[32] and 3) a focused library (conventional combinatorial chemistry).[33] A visual representation of the diversity of the collections in "chemical space" is depicted in Figure 1, and corresponding data are given in Table 1. Describing each compound by a series of physicochemical properties, followed by principal component analysis (PCA), enables quantitative estimation of the diversity achieved on a per-compound basis. Using this dataset, the DOS library, numerically, is even more diverse than the MDDR compounds, that is, 22 (relative) units for the DOS library, 19 for

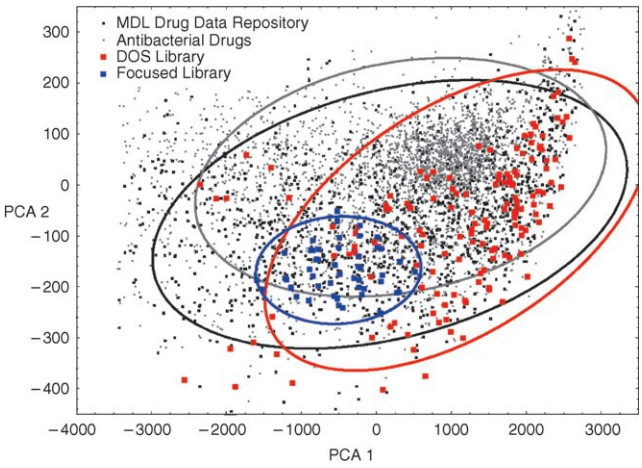


Figure 1. Visual representation of the diversity of different chemical collections in physicochemical and topological space using 184 molecular operating environment (MOE) descriptors followed by principal component analysis (PCA). The DOS library synthesized in this work is depicted by red squares. For comparison, a focused library (blue squares), the MDL Drug Data Repository (MDDR, black dots), and antibacterial drugs (gray dots) are depicted.

Table 1: Diversity of different chemical collections.

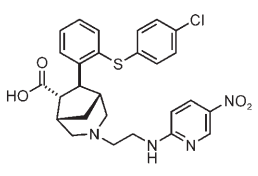
Library (MW<650)	σ[a] (PC1)	σ (PC2)	σ (PC3)	Average chemical space occupied per compound
MDDR	1472.11	122.53	104.56	18.86
antibacterials	1276.50	109.01	91.92	12.79
DOS	1176.97	135.69	139.78	22.32
focused	473.57	43.91	31.00	0.64

[a] Standard deviation of each representative molecular dataset in descriptor space.

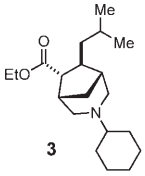
the MDDR, 13 for the antibacterials, and 0.6 for the focused library (Table 1). The DOS library spans a large part of biologically relevant chemical space and within this context is more diverse than the MDDR library, thus illustrating the value of our approach to deliver distinct products within the molecular diversity spectrum.[15]

The library compounds were screened for their effect on the growth of three strains of *S. aureus*: a methicillin-susceptible *S. aureus* (MSSA) and two UK epidemic methicillin-resistant strains (EMRSA 15 and EMRSA 16).[34] Both MRSA strains are resistant to penicillins and erythromycin and are responsible for the majority of infections with MRSA in the UK.[35] Three compounds reproducibly prevented the growth of these *S. aureus* strains and were rescreened to establish the lowest concentration at which they prevented growth (Table 2). The most potent compound, which we have called gemmacin, was investigated further. Structure–activity relationship (SAR) analyses demonstrated that both enantiomers had similar potency (Table 2). The enantiomers do not map onto the same pharmacophore, suggesting that their mode of action may not involve a protein active site or receptor. However, it was also notable that most structural changes to gemmacin (e.g. ethyl ester or remove NO_2) resulted in significant loss of activity (13 analogues were

Table 2: Structure and activity (MIC_{50}) of the three compounds identified from the diversity-oriented synthesis with growth inhibitory activity against three strains of *S. aureus*. ND = not determined.



(−)-gemmacin



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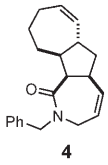

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	MIC_{50} [μg mL^{-1}] MSSA	EMRSA 15	EMRSA 16
(±)gemmacin	2	16	32
(−)gemmacin	ND	8	16
(+)gemmacin	ND	16	32
(±)-**3**	16	16	>64
(±)-**4**	32	32	>64
erythromycin	0.5	>64	>64
oxacillin	0.5	>32	>32

made; see the Supporting Information, section 15). Gemmacin showed broad-spectrum Gram-positive antibacterial activity in vitro, including inhibition of growth of vancomycin-intermediate *S. aureus* and vancomycin-resistant enterococci (VRE; see the Supporting Information, section 17). Gemmacin was not generally active against Gram-negative organisms, although it showed activity against two strains of *Moraxella catarrhalis* ($MIC = 16\ \mu g\,mL^{-1}$, where MIC is the minimum inhibitory concentration). The compound is a selective antibacterial agent, as it showed low antifungal activity ($MIC > 64\ \mu g\,mL^{-1}$ for seven *Candida* species) and low mammalian cell toxicity ($IC_{50} > 64\ \mu g\,mL^{-1}$ in human epithelial cells).

Target identification of (−)-gemmacin was attempted with assays used to identify common antimicrobial modes of action (such as dihydrofolate reductase inhibition, protein synthesis, and ATP synthesis uncoupling; gemmacin was inactive in all of these assays). Gemmacin did show activity in an assay used to detect the generation of reactive oxygen species ($IC_{50} = 0.35\ \mu g\,mL^{-1}$ in *Spodoptera frugiperda* cell line 21), which suggested to us that gemmacin may be a cell-membrane disrupter. This mode of action is consistent with the assay shown in Figure 2.

EMRSA 16 samples were inoculated with sub-lethal and lethal doses of gemmacin. Controls, containing dimethylsulfoxide (DMSO) and only the bacteria, were used for a direct comparison. Cells incubated with gemmacin did not show lysis of *S. aureus*, owing to the thick cell wall. The cells were then treated with lysostaphin,[36] an enzyme that cleaves the cross-linking pentaglycine bridges in the cell wall of staphylococci. This procedure leaves the cell membranes intact but reduces the optical density of the samples, as the "wall-less" cells are less turbid. However, in the presence of lethal doses of gemmacin, the membranes appear to have been disrupted partially, and without the protection of the cell wall many cells lyse, as detected by a more pronounced reduction in optical density. When the samples are treated with the detergent sarkosyl, membranes are disrupted, the cells lyse, and turbidity is lost completely. While selective membrane

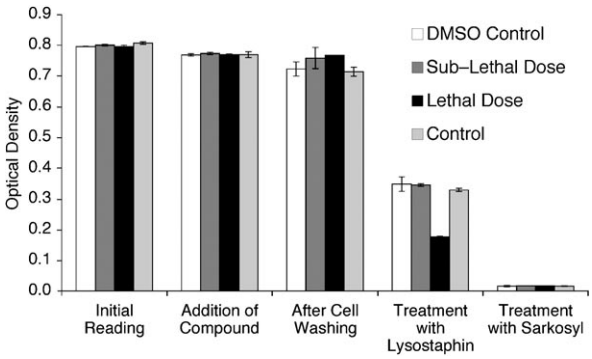

Figure 2. Membrane disruption assay. Samples of EMRSA 16 were analyzed which had been treated with DMSO (DMSO control), 4 μg mL^{-1} gemmacin (sub-lethal dose), 34 μg mL^{-1} gemmacin (lethal dose), and nothing (control). The incubated samples did not show lysis of *S. aureus* (as evidenced by the same optical density of samples) owing to the intact cell wall. When the pentaglycine crosslinks in the cell wall were cleaved by lysostaphin, the optical density of the samples is reduced. However, the sample treated with a lethal dose of gemmacin resulted in a more pronounced reduction in optical density, indicative of membrane disruption. Finally, the samples were treated with the detergent sarkosyl, which disrupts *S. aureus* cell membranes, resulting in complete cell lysis.

disruption of bacterial cells may not be the only mechanism of action of gemmacin, it is interesting to note that this is the primary mode of action of antimicrobial peptides such as magainins, defensins, gramicidin S, type A lantibiotics, and telavancin,[1] which all have molecular weights greater than 1700 g mol^{-1}. It is intriguing that the significantly smaller molecule gemmacin (539 g mol^{-1}) could have a similar mode of action, and that its activity is so sensitive to structural changes. However, it should be noted that in the field of agrochemicals, selective membrane disruption is the mode of action of several small-molecule herbicides such as acifluorfen and sulfentrazone.

In summary, we have described a divergent synthetic strategy that has been exploited in antibiotic discovery. The aim of diversity-oriented synthesis is to achieve efficiently high levels of skeletal diversity to explore biologically relevant regions of chemical space. A collection of 242 natural-product-like and drug-like small molecules was synthesized, which was more physicochemically and topologically diverse than databases of known drugs. Antibacterial screening with pathogenic strains of MRSA uncovered several hits, including gemmacin, which is likely to be a selective bacterial membrane disrupter. The unusual molecular scaffold and unique structural features, together with the positive in vitro results against MRSA strains and other bacterial pathogens, highlight that gemmacin provides a new structure for the discovery of critically needed antibiotics. Moreover, the discovery of gemmacin serves to endorse the diversity-oriented synthetic approach as a way to discover new classes of small molecules with desired biological activity.

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