Biocatalytic Construction of Quaternary Centers by Aldol Addition of 3,3-Disubstituted 2-Oxoacid Derivatives to Aldehydes

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ABSTRACT: The congested nature of quaternary carbons hinders their preparation, most notably when stereocontrol is required. Here we report a biocatalytic method for the creation of quaternary carbon centers with broad substrate scope, leading to different compound classes bearing this structural feature. The key step comprises the aldol addition of 3,3-disubstituted 2-oxoacids to aldehydes catalyzed by metal dependent 3-methyl-2-oxobutanoate hydroxymethyltransferase from *E. coli* (KPHMT) and variants thereof. The 3,3-trisubstituted 2-oxoacids thus produced were converted into 2-oxolactones and 3-hydroxy acids and directly to ulosonic acid derivatives, all bearing *gem*-dialkyl, *gem*-cycloalkyl, and spirocyclic quaternary centers. In addition, some of these reactions use a single enantiomer from racemic nucleophiles to afford stereopure quaternary carbons. The notable substrate tolerance and stereocontrol of these enzymes are indicative of their potential for the synthesis of structurally intricate molecules.

INTRODUCTION

Quaternary centers are important structural units that can be found in naturally occurring, biologically active compounds and pharmaceutical ingredients (Figure 1).¹ Bioactive molecules bearing this feature are becoming most appreciated in medicinal chemistry because they (i) expand the chemical

diversity besides the heteroaromatic “flat” chemistry, (ii) enhance the binding selectivity with target proteins avoiding unspecific interactions (e.g., π -stacking), and (iii) have better solubility and bioavailability.²

Therefore, synthetic access to this type of compound can be regarded as significant and a challenging task for organic chemists. Indeed, creation of quaternary stereocenters is elusive mainly due to the steric hindrance between the carbon substituents, and consequently, considerable efforts have been dedicated to implement chemical methodologies for this purpose.^{1a,g,3} These encompass long-established approaches such as Claisen rearrangement⁴ and Diels–Alder reaction⁵ up to the most recent ones based on transition metal catalysis^{1c,2b,3e,6} and organocatalytic reactions.^{3k,7} However, innovative procedures expanding the range of structurally diverse products with improved efficacy, simplicity, and stereoselectivity are key for advancing the field of chemical synthesis.^{6c,8}

Biocatalytic creation of carbon–carbon bonds is widely recognized as a useful synthetic technology particularly because of its high selectivity, mild operation conditions, and use of simple starting materials in a straightforward fashion.⁹

Figure 1. Examples of bioactive molecules and pharmaceuticals bearing quaternary centers.

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46 Furthermore, biocatalysis is accepted as a green and sustainable
 47 technology.¹⁰ However, enzymatic methods to forge quater-
 48 nary carbon stereocenters are scarce.¹¹
 49 In this work, we explored the capabilities of metal-dependent
 50 3-methyl-2-oxobutanoate hydroxymethyltransferase (KPHMT,
 51 EC 2.1.2.11) from *E. coli* and its variants to catalyze the
 52 formation of quaternary centers. In vivo, KPHMT catalyzes the
 53 transfer of the hydroxymethyl group from *N*⁵,*N*¹⁰-methylene
 54 tetrahydrofolate (MTHF) to 2-oxoisovalerate (2-OI) to
 55 produce ketopantoate (KP), bearing a *gem*-dimethyl quater-
 56 nary center and tetrahydrofolate (THF) (Scheme 1).¹² It has

Scheme 1. 3-Methyl-2-oxobutanoate Hydroxymethyltransferase (KPHMT) Catalyzed the Synthesis of Ketopantoate (KP), a Precursor of Vitamin B5^a

^aMTHF = *N*⁵,*N*¹⁰-methylene tetrahydrofolate; THF = tetrahydrofo-
 late. CoASH = Coenzyme A.¹⁴

57 also been found that KPHMT can function as a metal-
 58 dependent (Class II) aldolase, catalyzing the aldol addition of
 59 2-oxoacids to methanal and operating under a tetrahydrofolate-
 60 independent mechanism.¹³ Indeed, the enzyme is able to
 61 catalyze the enolization of the 2-oxoacid via an α -proton
 62 abstraction and formation of the corresponding stabilized
 63 carbanion. This enolate can attack the methylene group of
 64 MTHF, resulting in the formation of KP, in vivo activity, or
 65 eventually on other electrophiles, e.g., aldehydes.^{13a}

66 ■ RESULTS AND DISCUSSION

67 In a previous work, we reported on the preparation of 2-
 68 substituted 3-hydroxycarboxylic esters from 3-monosubstituted
 69 2-oxoacids and methanal.^{13b} Here, we investigated the aldol
 70 addition of 3,3-disubstituted 2-oxoacids (1) to diverse
 71 aldehyde (2), mediated by KPHMT catalysts, to generate
 72 3,3,3-trisubstituted 2-oxoacids (3) bearing a quaternary
 73 stereocenter (Scheme 2).

74 To establish optimal KPHMT catalysts, we capitalize on the
 75 recently engineered KPHMT variants I212A and I202A, which
 76 tolerate 3-monosubstituted 2-oxoacids with linear, branched,
 77 or cyclic substituents.^{13b} Furthermore, based on the KPHMT
 78 structure (Figure 2A), we envisioned that residues I212, I202,
 79 as well as V214 could also be involved in the accommodation
 80 of the tertiary carbon nucleophiles (i.e., 3,3-disubstituted 2-
 81 oxoacids).^{13b} Molecular models of variants at these positions
 82 showed larger active sites that could accommodate the
 83 sterically hindered nucleophiles considered (Figure 2 and

Scheme 2. Selected Panel of 3,3-Disubstituted 2-Oxoacids (1) and Aldehydes (2) as Substrates for KPHMT-Catalyzed Formation of Quaternary Carbon Centers by Aldol Addition Reactions

Figures S117–S128). Thus, seven KPHMT variants, i.e., 84
 I212A, I202A, V214G, I202A/I212A, I202A/V214G, I212A/
 85 V214G, and I202A/I212A/V214G, were constructed and
 86 tested as biocatalysts. Notably, the enzymes were obtained in
 87 high yields after expression and purification (88–205 mg of
 88 protein per liter of medium culture, see SI). Purified KPHMT
 89 catalysts were stored and used in the reactions as glycerinate
 90 preparations (i.e., a solution of the enzyme consisting of 20
 91 mM MOPS buffer pH 7.0 containing NaCl (50 mM) and
 92 glycerol (50%)). Furthermore, we previously found that
 93 KPHMT catalysts with Co²⁺ as metal cofactor gave the best
 94 activity.^{13b} Therefore, we decided to employ Co²⁺ as a cofactor
 95 for all KPHMT catalysts in this work.

Screening of KPHMT Catalysts and Substrates. We
 97 began our investigation on the screening of the KPHMT
 98 catalysts assaying the panel of nucleophiles and electrophiles
 99 for measuring the formation of aldol products 3 and/or
 100 consumption of substrates after 24 h (Figure 3, see SI) and
 101 reaction rates (v_0) (Figures S74 and S75).

Despite that wild-type KPHMT efficiently catalyzed the
 103 aldol reaction in some cases, variants I202A, I212A, and the
 104 double variant I202A/I212A gave the best ($\geq 90\%$) con-
 105 versions for most of the reactions and variant V214G
 106 specifically for the addition of 1a to 2h. Several KPHMT
 107 variants gave conversions $\geq 60\%$ with combinations of
 108 nucleophiles 1a, 1e, and 1f with electrophiles 2a–h.
 109 Exceptions were 2b+1f (50%) and 2d+1f (28%). 2-Oxoacids
 110 1g and 1h were substrates when methanal (2a) (20 and 40%
 111 with variants I212A and I202A/I212A, respectively) and 2-
 112

Figure 2. (A) Structure of the active site of the ketopantoate complex of wild-type KPHMT (chain A, PDB 1M3U).¹⁵ Ketopantoate is shown with yellow C atoms, and the three residues proposed for mutation are shown with orange C atoms. Models of the prereactive complexes of (B) KPHMT V214G with (S)-1b and 2a, (C) KPHMT I202A/V214G with (S)-1d and 2a, and (D) KPHMT I212A with 1f and 2h. Nucleophiles 1b, 1d, and 1f are shown with yellow C atoms, electrophiles 2a and 2h with green C atoms, and the mutated residues with orange C atoms. Nucleophiles are shown in their enolate form (1b and 1d as *E*-enolates) coordinated to the metal cofactor (purple sphere), which is also coordinated by residues D45 and D84 and by two water molecules. 2a and 2h are displayed with their carbonyl oxygen atom forming hydrogen bonds with residue E181 and one of the metal-coordinated waters. 2h is shown approaching the enolate from its *si*-face. The surfaces of the active site cavities near the nucleophile substrates are shown in cyan. Interactions are shown with dashed lines: H-bond in yellow, salt bridges in magenta.

E		2a													
KPHMT	Nu	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k	1l	1m	1n
wild-type		95	41	8	— ^a	53	49	— ^a	5	29	84	10	15	— ^a	— ^a
I212A		71	47	— ^a	— ^a	63	67	20	12	14	15	12	7	— ^a	— ^a
I202A		91	67	37	13	82	87	— ^a	10	55	95	25	20	— ^a	— ^a
V214G		90	41	10	8	47	16	— ^a	10	14	80	11	6	— ^a	— ^a
I202A/V214G		75	46	15	23	43	49	— ^a	5	15	68	14	7	— ^a	— ^a
I212A/V214G		25	16	5	— ^a	30	22	— ^a	—	6	22	12	8	— ^a	— ^a
I202A/I212A/V214G		18	14	— ^a	10	31	54	— ^a	5	7	18	12	5	— ^a	— ^a
I202A/I212A		63	35	10	7	72	90	8	40	18	50	26	16	— ^a	— ^a

E		2b			2c			2d			2e						
KPHMT	Nu	1a	1e	1f	1a	1e	1f	1g	1h	1a	1e	1f	1a	1e	1f	1g	1h
wild-type		87	90	41	80	95	60	— ^a	— ^a	44	42	10	84	86	32	— ^a	— ^a
I212A		70	90	50	82	91	93	50	42	37	60	20	85	97	75	— ^a	— ^a
I202A		79	91	41	88	96	93	— ^a	— ^a	43	46	25	85	95	49	— ^a	— ^a
V214G		78	86	30	80	60	20	— ^a	— ^a	42	29	— ^a	80	61	20	— ^a	— ^a
I202A/V214G		73	83	35	77	43	40	— ^a	14	35	30	5	62	44	27	— ^a	— ^a
I212A/V214G		53	71	38	18	90	38	— ^a	14	4	41	5	33	72	33	— ^a	— ^a
I202A/I212A/V214G		22	54	48	12	40	70	— ^a	21	2	21	5	5	38	30	— ^a	— ^a
I202A/I212A		48	90	40	68	93	85	— ^a	— ^a	12	54	28	82	95	75	— ^a	— ^a

E		2f			2g			2h						
KPHMT	Nu	1a	1e	1f	1a	1e	1f	1g	1h	1a	1e	1f	1g	1h
wild-type		87	80	26	82	98	63	— ^a	— ^a	60	97	— ^a	— ^a	— ^a
I212A		73	95	82	79	98	91	— ^a	— ^a	42	98	64	— ^a	— ^a
I202A		90	95	65	80	98	67	— ^a	— ^a	73	61	— ^a	— ^a	— ^a
V214G		67	63	12	81	97	31	— ^a	— ^a	90	91	— ^a	— ^a	— ^a
I202A/V214G		43	40	20	74	53	40	— ^a	— ^a	33	27	— ^a	— ^a	— ^a
I212A/V214G		32	83	20	47	93	80	— ^a	— ^a	15	98	39	— ^a	— ^a
I202A/I212A/V214G		27	39	27	30	91	67	— ^a	— ^a	26	53	— ^a	— ^a	— ^a
I202A/I212A		67	96	80	79	98	90	— ^a	— ^a	26	98	30	— ^a	— ^a

Figure 3. Screening of KPHMT-catalyzed aldol addition of selected 3,3-disubstituted 2-oxoacids 1 to aldehydes 2. Conversions to aldol adducts (%) at 24 h of reaction measured by HPLC after derivatization of the substrates and products via oxime formation (see SI). Other nucleophile/electrophile combinations were not performed due to the low activity in the screening with methanal as electrophile (Figures S74 and S75). ^aNo product formation was detected.

hydroxyethanal (2c) (50 and 42%, respectively, with I212A) were used as electrophiles, whereas they were not converted using 2b and 2e–h. Nucleophiles 1m–n and electrophiles 2i–k were not converted by any KPHMT catalysts. The results are consistent with the initial reaction rates (v_0) of the corresponding reactions (Figures S74 and S75). Notably, KPHMT(Co^{2+}) catalyst loading was 0.068 mol %. This catalytic efficiency in addition to their high expression levels make these enzymes highly valuable for preparative-scale synthesis.

Preparative Synthesis. The positive reactions were run at a preparative scale (2.5 mmol of the limiting 3,3-disubstituted 2-oxoacid (1) substrate), providing, after the adequate treatment (see below), the corresponding 2-oxolactones (4a–g) (Scheme 3A), 3-hydroxy acid derivatives (5a–j) (Scheme 3B), bicyclic lactones (6) (Scheme 4A), and ulosonic acid type products (7a–q) (Scheme 4B), all of them bearing *gem*-dialkyl, *gem*-cycloalkyl, and spirocyclic quaternary centers, with final product amounts ranging from 50 to 390 mg. The 2-oxolactones 4a–g were produced during workup (acidic aqueous–organic solvent extraction). Thus, when quantitative conversions were achieved, this methodology was enough to afford pure material (Scheme 3A). Otherwise, an additional extraction with aqueous buffer at neutral pH removed the remaining starting 2-oxoacid 1, which was the main impurity present. The 2-oxo group can be reduced,¹⁶ affording α -hydroxy- γ -butyrolactones (e.g., (*R*)- or (*S*)-pantolactone and

analogues), chiral auxiliaries, and important precursors for the synthesis of naturally occurring and synthetic biologically active compounds.¹⁷ Elaborated chemical transformations on the carboxylate group such as oxidative decarboxylation followed by esterification lead to different 3-hydroxyesters (5a–i and amide 5j, Scheme 3B) with application as building blocks in the synthesis of pharmaceutical compounds¹⁸ and polymers, coating oligomers, and dendrimers, e.g., 2,2-bis(hydroxymethyl) propionic acid derivative 5g.¹⁹ Using 2-hydroxyaldehydes, 2c and 2e–h, as electrophiles, the spontaneous formation of cyclic hemiketals effectively shifted the reaction equilibrium to the aldol products 6a,b and 7a–q (Scheme 4A,B). The resulting adduct products were easily purified by anion exchange chromatography, eluting with formic acid. These ulosonic acid type products, bearing a quaternary center at C3, have not been hitherto reported, and their preparation using conventional chemical procedures may be anticipated to be elusive because of the number of functional groups and the intrinsic reactivity of the 2-oxoacid moiety.²⁰ Furthermore, KPHMT would facilitate access to a great variety of ulosonic acids (e.g., sialic acid family), which constitute important biologically active compounds involved in cellular recognition and communication.²¹

The addition of 1j to 2c (Scheme 4A) gave a mixture of the two diastereomeric lactones 6a and 6b (91:9, respectively), identified by NMR after anion exchange purification. The acidic elution conditions (i.e., HCOOH , 1 M) and the

Scheme 3. General Routes for the Synthesis of Products 4 (A) and 5 (B)

^aI202A. ^bAbsolute stereochemistry assigned on the basis of crystal X-ray diffraction of compound 5c. ^cI202A/V214G. ^dI212A. ^eNot determined. For optical rotations see SI. ^fWild-type. ^gEnantiomeric ratio determined by NMR using *R*-configured Pirkle's alcohol.²² ^hEnantiomeric ratio determined by HPLC on the chiral stationary phase. ⁱV214G. ^jThe absolute stereochemistry was determined by single-crystal X-ray diffraction (see SI). ^kI202A/I212A. ^{a,c,d,f,i,k}Catalyzed by 0.068 mol % of KPHMT(Co²⁺) wild-type or variants thereof.

lyophilization of the fraction pool promoted the lactonization of the hemiketal intermediate.

The absolute stereochemistry of 4f–g and 7a–e was assigned by comparing the stereochemical outcome achieved for 7f–q. Compounds 7f–q contain one or two chiral centers of defined absolute configuration that act as a reference in the determination of their overall relative configuration. In 7f–q the absolute stereochemistry of the chiral center coming for the electrophile was invariably *S* regardless of the aldehyde substrate. This is also consistent with a previous study on the steric course of KPHMT-catalyzed aldol reaction with MTHF.²³ Therefore, it may safely be assumed that the major enantiomer of 4f–g, 7a–e, and 5h will be *S*, and that of 5j will be *R* configured. The absolute stereochemistry of 5b was assessed indirectly by analyzing the stereochemistry of the unreacted 1b. This was determined to be *R* configured according to chiral HPLC analysis against a standard (see Figure S77). This indicated that only the corresponding enantiomer *S*-1b was converted. From mechanistic considerations of KPHMT catalysis (see below and Scheme 5), it may be inferred that the *S*-1b furnished *S*-5b. The X-ray

diffraction provided the absolute *S*-configuration of 5c (Figure S79) that agrees with the *S*-5b stereochemistry.

The *S*-configured 5b and 5c could be rationalized based on the molecular models shown in panels B and C of Figure 2. To form the corresponding enolates, nucleophiles 1 should adopt a bound orientation in the catalytic site with their H atom at C3 oriented toward the proposed catalytic base (E181).¹⁵ Therefore, it could be reasoned that the *S*-enantiomer of 1b can adopt an appropriate conformation with minimal steric repulsion for its C3-ethyl substituent (Scheme 5). The corresponding *E*-enolate would then expose its *re* face to the electrophile (Figure 2B), providing the 3*S*-aldol adduct, a precursor of *S*-5b. In contrast, *R*-1b has to adopt a different bound conformation, with its C3-ethyl substituent pointing toward residue V179, in order to place its α -proton within reach of the catalytic base (Scheme 5). This conformation, which is less favored because of the proximity of the ethyl moiety to the side chains of V179 and E181, would generate a *Z*-enolate that would expose its *si*-face to the electrophile, providing the alternative 3*R*-aldol adduct. Similarly, each enantiomer of nucleophile 1d can only adopt one reactive conformation that would generate the corresponding *E*- and *Z*-

Scheme 4. General Routes for the Synthesis and Isolation of Products 6 and 7

^aMeasured by NMR. ^bNot determined. For optical rotations, see SI. ^cNo other diastereomers were detected by NMR analysis. ^d α : β anomeric ratio. ^{e-h}Catalyzed by 0.068 mol % of: ^ewild-type KPHMT(Co²⁺), or variants ^fI202A, ^gI212A, ^hV214G.

Scheme 5. Enolization Mechanism of Nucleophiles S-1b,d and R-1b,d

210 enolates (Figure 2C), from S- and R-1d, respectively. Due to
 211 the larger size of the *n*-butyl C3-substituent, formation of the
 212 *E*-enolate would be faster, and consequently, the 3*S*-aldol
 213 adduct is formed preferentially. Similar reasoning could be
 214 used to explain the steric outcome observed for the rest of the
 215 products (see SI).

In some cases, minor cyclic structures such as 2-oxolactone **8** 216
 and the corresponding hydrated form **9** (Scheme 6 and spectra 217 s6
 in SI) were identified. It is likely that the purification process 218
 under acid conditions and freeze drying favored the formation 219
 of these side derivatives. 220

The addition of **1a**, **1e**, and **1f** to D-threose was planned as a 221
 simultaneous biocatalytic one-pot two-step cascade reaction 222 s7

Scheme 6. Equilibrium between Lactones 8 and 9 and Hemiketal 7 in Acidic Aqueous Medium

oxoacid nucleophiles and aldehyde substrates. The biocatalytic approach reported here benefits from a straightforward process starting from diverse 2-oxoacid derivatives, obtained by well-established and efficient chemical procedures. This methodology allows access to unprecedented 2-oxolactones, which can be converted into α -hydroxy- γ -butyrolactones by reduction of the carbonyl group, 3-hydroxy acid derivatives, and ulosonic acid type products, all of them bearing *gem*-dialkyl, *gem*-cycloalkyl, and spirocyclic quaternary centers. To the best of our knowledge, the aldol additions of 3,3-disubstituted 2-oxoacid derivatives to aldehydes have not been performed with other catalysts. For example: the enamine activation of branched α,α -disubstituted carbonyl compounds via organocatalysis (e.g., proline and derivatives using typically 1–20 mol % compared with KPHMT(Co^{2+}) catalysts employing 0.068 mol %) is much less reactive with respect to those derived from α -monosubstituted carbonyl compounds, and therefore only very strong electrophiles are able to react.^{3a} Here, we are using common aldehydes not specially activated and some even in equilibrium with cyclic hemiketals in aqueous solution, e.g., D-threose and D-erythrose.^{3a} The present study expands the portfolio of biocatalytic aldol reactions leading to unprecedented structures and offering new synthetic opportunities for pharmaceutically relevant compounds and chiral precursors. We anticipate a range of new applications by performing further engineering of the KPHMT variants to render new biocatalysts, efficient toward new substrate classes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.0c09994>.

Crystallographic data for structure 5c (CIF)

Experimental details, NMR spectra, and computational models (PDF)

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Scheme 7. One-Pot Two-Step Enzymatic Synthesis of Products 7l (A) and the Diastereomeric Mixture 6a:6b (B)^a

^aMBP-YfaU = maltose binding protein fused to YfaU.^{13b}

■ CONCLUSION

In summary, we demonstrate the utility of the promiscuous 3-methyl-2-oxobutanoate hydroxymethyltransferase (EC 2.1.2.11) (KPHMT), for the creation of quaternary carbon centers using a structurally diverse array of 3,3-disubstituted 2-

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311 Author Contributions

312 [†]R.M.-V. and K.H. contributed equally. The manuscript was
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315 Notes

316 The authors declare no competing financial interest.

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