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Development of scalable precision fermentation and chemical-grade purification of 5-aminovaleric acid for high-value valorization

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ABSTRACT

The use of biogenic 5-aminovaleric acid (5-AVA), a key bio-monomer offers a plausible pathway for transforming the petrochemical industry into a low-carbon emission sector. However, its broader industrial application is limited by the absence of established precision fermentation platforms that yield high 5-AVA titers. Scalable downstream purification strategies for its purification are also lacking. Therefore, to address these issues, this study presents a precision fermentation platform for the high-precision synthesis of 5-AVA using an engineered *Corynebacterium glutamicum* strain, AVA-3. Via stepwise metabolic engineering to enhance l-lysine flux and suppress byproduct formation, we obtained the highest 5-AVA titers reported to date: 51.8 at 5 L (lab scale) and 44.4 g/L at 500 L (pilot scale). Tailored media supplemented with polypeptone and trace elements improved cell growth and 5-AVA yield, highlighting the effectiveness of precision fermentation in scalable bioprocess development. Additionally, a downstream purification protocol for obtaining high-purity 5-AVA, which showed a recovery rate of approximately 50 %, was also established. Further experiments indicated that the high-purity 5-AVA thus obtained could serve as a renewable platform chemical as it could be used as a precursor in the production of bio-based nylon. Representative chemical valorizations of bio-based 5-AVA also led to the formation of value-added compounds with potential for application in functional materials as precursors for antibody-drug conjugate linkers and metabolically important compounds. These findings position *C. glutamicum* AVA-3 as a robust microbial chassis for sustainable, industrial-scale 5-AVA production and may contribute to ongoing efforts toward establishing carbon-efficient biomanufacturing and a circular bioeconomy.

1. Introduction

Since 2020, the petrochemical industry, which accounts for 6.1 % of total CO_2 emissions, and contributes to a range of environmental issues, including climate change, has faced several major challenges in the context of carbon cycling [1]. The traditional industrialization model, which heavily relies on the petrochemical sector and emphasizes large-scale production and consumption, is a serious threat to the long-term survival of humanity. In response to these challenges, bioeconomy, which offers renewable and low-carbon alternatives across various petroleum-based industries, including plastics, chemicals, and pharmaceuticals, has emerged as a promising pathway for transforming the

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petrochemical industry into a low-carbon emission industry [2–4].

Precision fermentation represents a central technology driving this bioeconomic transition [5,6]. It combines microbial strain engineering, fermentation improvement, and downstream process integration to enable selective, efficient, and scalable production of target compounds from renewable resources such as biomass and plastic waste. Whereas conventional fermentation is the process that has focused on improving the productivity and growth of wild-type strains, precision fermentation is the process for the production of industrially useful products by employing the strategies including the development of metabolically engineered microbial strains having enhanced metabolic power by screening and implementing improved medium components and cultivation conditions that promote efficient production of the desired compound, thereby improving overall productivity [7,8]. In addition to its advantages, including the possibility to improve process yield and generate high-purity products, it also aligns industrial production with circular economy principles.

5-Aminovaleric acid (5-AVA), a C5 bio-based monomer with an amine group, is a key intermediate in the production of polyamides (e.g., nylon-5 and nylon-5,6) and polyesters, and over the years, it has attracted considerable attention as a sustainable alternative to petrochemical-derived monomers [8,9]. To enable scalable production and lower costs, recent findings recommend a shift to de novo biosynthesis from glucose using metabolically engineered *Corynebacterium glutamicum*, a high-flux industrial host. Specifically, employing the l-lysine-overproducing *C. glutamicum* BE strain harboring *davB* and *davA*, which encode lysine 2-monooxygenase and δ-aminovaleramidase, in fed-batch fermentation under the strong H36 promoter yielded 33.1 g/L of 5-AVA [10]. Further, cost reduction using *Miscanthus sacchariflorus* hydrolysate as a low-cost carbon source, yielded 12.51 g/L of 5-AVA from 298 g/L glucose [11]. To the best of our knowledge, the highest reported 5-AVA titer of 46.5 g/L was achieved by introducing *davB* and *davA* into the l-lysine-overproducing *C. glutamicum* LYS strain, deleting *gabT* and *argD*, which encode GABA transaminase and *N*-acetylornithine aminotransferase, respectively, and chromosomal integration of the 5-AVA exporter GabP-III, to enhance productivity and eliminate glutaric acid (GTA) byproduct formation [12].

However, the transformation of traditional petroleum-based industries into bio-based industries cannot be achieved solely via the development of efficient microbial production, which of course is important for realizing the full potential of bio-based manufacturing. A key challenge that needs to be addressed, particularly, in commercial bioprocesses, is downstream purification, which plays a critical role in determining both product quality and overall process cost. The industrial deployment of 5-AVA remains constrained by significant downstream purification costs, typically accounting for 50–70 % of total production expenses for highly polar and water-soluble molecules [13]. Its high water solubility, presence in complex fermentation broths, and chemical similarity to other organic acids and amino acids render conventional purification methods—such as solvent extraction or ion-exchange chromatography—inefficient, costly, and sometimes environmentally unsustainable [13–15]. Therefore, establishing scalable, economically feasible, and environmentally sustainable purification techniques not only ensures the effective removal of impurities and by products but also enhances compliance with regulatory frameworks and meets application-specific requirements. Developing such a purification method is also particularly important in bioplastic manufacture because monomer purity directly affects polymer performance [16,17].

Microbially synthesized 5-AVA has great potential as a bio-based monomer for the manufacture of plastics, such as nylon-6,5, and as a precursor for the synthesis of other monomers, such as 1,5-pentanediol, GTA, and 5-hydroxyvaleric acid [2,18,19]. However, its broader industrial use and integration into the growing bioeconomy is limited by the absence of established precision fermentation platforms for its production and underdeveloped downstream and transformation strategies. To address these challenges, in this study, we aimed to establish a scalable and economically viable 5-AVA production platform based on *C. glutamicum* PKC, an industrially optimized L-lysine-overproducing strain. A novel process for the scalable and high-purity recovery of 5-AVA was developed and novel applications of this bio-based monomer were also explored. The findings of this study may facilitate scalable and high-purity 5-AVA production and its subsequent chemical valorization, and thus, advance the establishment of a circular economy.

2. Materials and methods

2.1. Chemicals, plasmids, and bacterial strains

All the chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified. Methanol, ethanol, dichloromethane, toluene, n-hexane, and ethyl acetate were purchased from Samchun Chemicals (Gyeonggi-do, Korea). The strains and plasmids used are listed in Table 1, and unless otherwise specified, all DNA manipulations were performed using polymerases, ligases, and restriction enzymes purchased from New England Biolabs (Ipswich, MA, USA) in accordance to the standard protocols of the manufacturer. Polymerase chain reaction (PCR) was performed using a SimpliAmp Thermal Cycler (Applied Biosystems, Waltham, MA, USA). The primers used, as listed in Table S1, were synthesized by Cosmo Genetech (Seoul, Korea). *Escherichia coli* DH5α was used for plasmid construction. Transformation was followed by culturing overnight at 37 °C and 220 rpm in Luria-Bertani (LB) medium (comprising 10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter) supplemented with either kanamycin (Km, 30 μg/mL) or spectinomycin (Sp, 50 μg/mL), as appropriate for plasmid amplification. To develop the 5-AVA biosynthesis platform, *davB* gene was inserted into the pCES208H30 vector using *Bam*HI and *Not*I restriction sites. Subsequently, *davA* gene was fused with an N-terminal His_6-tag using the *Not*I restriction site. Thus, the pCES208H30DavBHisA plasmid was obtained. Further, to enhance 5-AVA production, pBL712H30DapB$_{mut}$ was constructed by inserting the gene into the pBL712H30 vector using the *Hin*dIII restriction site.

To block the GTA pathway, *gabT* in *C. glutamicum* PKC was deleted. First, the 500-bp upstream and downstream regions of *gabT* were amplified and inserted into the pK19mobSacB vector using *EcoR*I and *BamH*I restriction sites to generate pK19mobSacBΔ*gabT*. Thereafter, gene deletion was performed via *sacB* counter-selection [20,21].

Table 1
Strains and plasmids used in this study.

Strains and plasmids	Characteristics	References or source
Strains		
E.coli DH5α	*E. coli* K-12; F$^-$, Φ80d*lacZ*ΔM15, Δ(*lacZYA-argF*)U169, *deoR*, *recA1*, *endA1*, *hsdR17*(r_K^- m_K^+), *phoA*, *supE*44, λ$^-$, *thi*-1	Invitrogen
C. glutamicum PKC	Industrially suitable L-lysine overproducing strain	[19]
C. glutamicum AVA	*C. glutamicum* PKC derivative, △*gabT*	In this study
AVA-1	*C. glutamicum* PKC with pCES208H30DavBHisA	In this study
AVA-2	*C. glutamicum* AVA with pCES208H30DavBHisA	In this study
AVA-3	*C. glutamicum* AVA with pCES208H30DavBHisA and pBL712H30DapB$_{mut}$	In this study
Plasmids		
pK19mobSacB △*gabT*	pK19mobSacB derivative for in-frame deletion of *gabT*	In this study
pCES208H30DavBHisA	pCES208 derivative; P_{H30} Promoter, *Pseudomonas putida*. KT2440 *davBHisA* genes, Km^R	In this study
pBL712H30DapB$_{mut}$	pCES208 derivative; P_{H30} Promoter, *E. coli* mutant *dapB*(C115G, G116C) genes, Sp^R	In this study

Specifically, the pK19mobSacBΔ*gabT* plasmid was introduced into *C. glutamicum* PKC to induce first homologous recombination and *sacB* integration was confirmed via colony PCR. Positive colonies were cultured and plated on RG agar with 10 % sucrose to trigger the second recombination. Finally, colonies growing on sucrose but not on Km were selected, and *gabT* deletion was verified via PCR and sequencing.

2.2. Culture conditions

C. glutamicum PKC, an industrially suitable L-lysine overproducing strain, was transformed with plasmids via electroporation to generate recombinant strains. To prepare the seed culture, *C. glutamicum* strains were inoculated into 2 mL of RG medium (recovery growth medium, containing 40 g brain heart infusion, 10 g beef extract, 30 g D-sorbitol, and 10 g glucose per liter) supplemented with Km (20 μg/mL) or Sp (200 μg/mL), depending on the plasmid used. This step was followed by incubation for 24 h at 30 °C with shaking at 250 rpm. Next, portions of the seed culture were inoculated into 20 mL of CG50 medium (containing 15 g yeast extract, 15 g $(NH_4)_2SO_4$, 50 g glucose, 2.0 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.01 g $MnSO_4{\cdot}H_2O$, 15 g $CaCO_3$, 0.5 mg biotin, and 0.3 mg thiamine·HCl per liter) supplemented with the appropriate antibiotics in 250-mL baffled flasks followed by culturing at 30 °C for 120 h with shaking at 250 rpm.

2.3. Lab-scale (5 L) fermentation

Lab-scale batch fermentation was performed using a 5 L jar fermenter (MARADO-05D-PS, CNS, Daejeon, Korea). In brief, a preculture was prepared by inoculating the seed culture into 20 mL of CG50 medium in a 250 mL baffled flask followed by incubation at 30 °C for 12 h with shaking at 250 rpm. The culture medium was supplemented with or without 15 g/L of polypeptone depending on the experimental conditions. Then, for the batch fermentation, the preculture was added to a 1 L working volume of CG100 medium (containing 15 g yeast extract, 15 g $(NH_4)_2SO_4$, 100 g glucose, 2.0 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.01 g $MnSO_4{\cdot}H_2O$, 0.5 mg biotin, and 0.3 mg thiamine·HCl) at 10 % (*v*/v), yielding an initial optical density at 600 nm (OD600) of 1–2. The system was then maintained at 30 °C under shaking at 600 rpm. Further, the pH was maintained at 6.9 ± 0.25 using 30 % ammonium hydroxide and foam formation was controlled using Antifoam 204, while aeration was provided at 5 vvm. Fed-batch fermentation was also performed under the same conditions, and the feed solution contained 700 g glucose, 270 g $(NH_4)_2SO_4$, 0.25 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, and 0.01 g $MnSO_4{\cdot}H_2O$ per liter. Thus, the glucose concentration was maintained in the range 10–20 g/L throughout the fermentation process.

2.4. Pilot-scale (500 L) fed-batch fermentation

For the 500 L pilot-scale (KoBioTech, Incheon, Korea) fed-batch fermentation process, seed culture was initially prepared in 2 mL of RG medium, and thereafter, inoculated into a 250 mL baffled flask containing 20 mL of CG50 medium. This step was followed by culturing at 30 °C for 24 h under shaking at 250 rpm. All of the resulting preculture was then transferred to a 2 L flask containing 360 mL of CG50 medium and the mixture (total volume of 400 mL) was incubated for an additional 24 h. Subsequently, the resulting culture medium was scaled up to 50 L with a working volume of 20 L, based on inoculation at 10 % (*v*/v), followed by further culturing for 24 h. Finally, fermentation was performed in a 500 L fermenter using 180 L of CG100 medium supplemented with 15 g/L polypeptone. The pH of the culture medium was maintained between 6.8 and 7.0 using 30 % ammonium hydroxide, and foam formation was controlled using Antifoam 204. Additionally, when glucose consumption slowed down, an appropriate amount of 100 g/L yeast extract solution was supplemented to promote cell growth and enhance 5-AVA production. The feeding solution had the same composition as that used in the lab-scale process. Thus, the glucose concentration was also maintained in the range 10–20 g/L throughout the fermentation process.

2.5. Scalable 5-AVA purification process

Crude 5-AVA solution (500 mL, 37.1 g/L) was initially concentrated via rotary evaporation at 55 °C and 190 mbar in vacuo. Thereafter, 1 L of methanol was added to facilitate the precipitation of impurities. Thus, a brownish solid was formed, and was filtered off using a microfilter (diameter, 240 mm; pore size, ~8–12 μm; HD Micro, Seoul, South Korea). The resulting filtrate was further concentrated in vacuo via heating at 40 °C under 150-mbar pressure. Solid impurities were further removed by re-dissolving the concentrate in 500 mL of ethanol, followed by filtration and subsequent concentration again at 40 °C under 90–mbar pressure. For preliminarily purification to obtain 5-AVA, a flash column (methanol/dichloromethane = 95:5, *v*/v; silica gel, 230–400 mesh) was employed. The solution collected from the column was evaporated in vacuo and the resulting product purified via precipitation using a dual-solvent mixture (methanol/dichloromethane = 10:90, *v*/v). Finally, 5-AVA (9.13 g, 49.2 %) was obtained as a yellow powder. Spectroscopic analysis yielded the following data: ^{1}H NMR (300 MHz, D_2O): δ = 2.86 (t, 2H), 2.08 (t, 2H), 1.50 (m, 4H); ^{13}C NMR (75 MHz, D_2O): δ = 182.8, 39.1, 36.6, 26.4, 22.5.; MS (ESI-MS): *m/z* calcd. for $C_5H_{12}NO_2$ $[M+H]^+$: 118.09; found: 118.01.

2.6. Synthesis of 1,3-dihydro-1,3-dioxo-2H-isoindole-2-pentanoic acid (DDIP, compound 1)

As a proof-of-concept, 5-AVA (115 mg, 0.982 mmol) was reacted with phthalic anhydride (145 mg, 0.982 mmol, 1.0 equiv.) in a mixed solvent system (toluene/methanol = 1:1 *v*/v, 20 mL) containing triethylamine (14.9 μL, 0.1 equiv.) at 70 °C for 24 h. At the end of the reaction, the solvent was evaporated under a reduced pressure, and the residue was purified via silica gel column chromatography (n-hexane/ethyl acetate = 80:20, v/v, 230–400 mesh) to yield compound 1 as a white powder (163 mg, 67.3 %). The spectroscopic analysis of this compound yielded the following information: ^{1}H NMR (300 MHz, d_6-DMSO): δ = 12.10 (s, br, 1H), 7.86 (m, 4H), 4.56 (t, 2H), 2.23 (t, 2H), 1.57–1.50 (m, 4H); ^{13}C NMR (75 MHz, d_6-DMSO): δ = 174.7, 168.5, 134.9, 132.1, 123.5, 37.6, 33.5, 27.9, 22.2; MS (ESI-MS): *m/z* calcd. for $C_{13}H_{13}NO_4$ $[M-H]^-$: 246.08; found: 246.00.

2.7. Synthesis of 4-carboxy-N,N,N-trimethyl-1-butanaminium (CTMB, compound 2)

To further demonstrate the applicability of biogenic 5-AVA, a sample (117 mg, 1.00 mmol) was methylated via reaction with potassium bicarbonate (400 mg, 4.00 mmol) and iodomethane (311 μL, 5.00 mmol) in methanol (5 mL) at room temperature for 3 days. Following solvent removal under vacuum, the residue was dissolved in distilled water (2 mL) and conc. HCl (2 mL). The resulting acidic aqueous solution was then stirred for an additional 4 h, evaporated, and re-dissolved in methanol (5 mL). After the filtration of precipitated yellowish solids, the organic solution was evaporated in vacuo. Additional conc. HCl (2 mL) was added, and the mixture was heated at 50 °C and stirred for 12 h. The resulting solid product, compound 2, was then isolated via filtration, washed with distilled water, and dried in an oven (125 mg, 78.0 %). Its spectroscopic analysis yielded the following: ^{1}H NMR (300 MHz, d_6-DMSO): δ = 12.18 (s, br, 1H) 3.27 (m, 2H), 3.02 (s, 9H), 2.30 (t, 2H), 1.68 (m, 2H), 1.49 (m, 2H); ^{13}C NMR (75 MHz, d_6-DMSO): δ = 174.5, 65.4, 52.6, 33.3, 22.1, 21.8; MS (ESI-MS): *m/z* calcd. for $C_8H_{18}NO_2$ $[M]^+$: 160.13; found: 160.13.

2.8. Analytical procedures

Cell growth was monitored by measuring OD600 using a spectrophotometer (UV-1900i Plus, Simadzu, Japan). The analysis of metabolites, including 5-AVA, l-lysine, and GTA from both flask cultures and fermentation processes, was realized via high-performance liquid chromatography (HPLC). Derivatization was performed prior to analysis using a diethyl ethoxymethylenemalonate (DEEMM) buffer system with composition of as follows: DEEMM, 6 μL; 240 mM methanol, 2 μL; borate buffer (pH 9.0), 600 μL; and distilled water, 292 μL. First, culture broth samples were subjected to centrifugation after which supernatant samples were collected, diluted with distilled water, and derivatized by rapidly adding 900 μL of DEEMM buffer to 100 μL of the diluted sample followed by incubation at 70 °C for 2 h. Next, 5-AVA and L-lysine contents were analyzed using the Agilent Infinity 1260 system (Agilent Technologies, Santa Clara, CA, USA) equipped with a Capcellpak C18 UG column (4.6 mm I.D. × 250 mm, 5 μm particle size). The mobile phase comprised acetonitrile (solvent A, 100 %) and 25 mM sodium acetate buffer (solvent B, pH 4.8). Gradient elution was performed at a flow rate of 1 mL/min with the column oven temperature maintained at 35 °C. The initial gradient program ratio (A:B) was 20:80, and after 2 min, the ratio of solvent A was increased to 25 %, then gradually to 60 % from 2 to 32 min, followed by a decrease back to 20 % by 40 min. Glucose and GTA contents were analyzed using a Shimadzu SCL-40 HPLC system (Shimadzu, Kyoto, Japan) equipped with an Aminex HPX-87H column (300 × 7.8 mm, 9 μm, Bio-Rad, Hercules, CA, USA). The mobile phase consisted of 5 mM H_2SO_4, and the analysis was performed at a flow rate of 0.8 mL/min with the column oven temperature maintained at 50 °C. To determine the structures of the chemical reaction products derived from 5-AVA, ^{1}H NMR, ^{13}C NMR, and electrospray ionization mass spectrometry (HR-ESI-MS) were employed. The NMR spectra were recorded in deuterated solvents using a 300-MHz spectrometer, (Bruker Daltonics, Billerica MA, USA), while ESI-MS analysis was performed using an exactive mass spectrometer (Waters, Milford MA, USA).

3. Results and discussion

3.1. The 5-AVA biosynthetic strain as a basis for precision fermentation

To develop a stable and efficient platform for 5-AVA production via precision fermentation, a previously reported biosynthetic pathway [19] (Fig. 1) was introduced into l-lysine-overproducing *C. glutamicum* PKC and thereafter, the 5-AVA production capability of the strain was verified [22] (Fig. 2a). The employed biosynthetic pathway comprises two key metabolic engineering strategies. The first is the blocking of 5-AVA conversion to the by product GTA as previously demonstrated in recombinant *C. glutamicum* BE (KCTC 12390BP) [10], and second is the enhancement of l-lysine overproduction by introducing an NADH-preferring DapB mutant, a strategy that has been successfully implemented in *C. glutamicum* PKC [19]. The initial strain for precision fermentation, *C. glutamicum* AVA-1, was constructed by introducing pCES208H30DavBHisA into *C. glutamicum* PKC. Prior to batch fermentation, the colony with the highest 5-AVA production capacity was selected via flask-level screening. After flask cultivation in CG50 medium at 30 °C under shaking at 250 rpm for 120 h, the 5-AVA titer of the selected colony was 9.84 g/L, while GTA accumulation reached 1.70 g/L (Fig. S1a). Further, under batch fermentation conditions (30 °C, 600 rpm, and 5 vvm) in CG100 medium, the 5-AVA titer of *C. glutamicum* AVA-1 was 5.0 g/L, with the OD600 value reaching 70.0 after 24 h of cultivation (Fig. S1b). However, significant GTA accumulation (11.6 g/L) was observed possibly owing to the activities of endogenous GabT (5-aminovalerate transaminase) and GabD (glutarate semialdehyde dehydrogenase) enzymes, which catalyzed 5-AVA degradation, consistent with the findings of a previous study using recombinant *C. glutamicum* BE (KCTC 12390BP) [10].

To enhance 5-AVA yield and reduce GTA formation, we deleted the *gabT* gene, generating *C. glutamicum* PKC Δ*gabT*, and introduced pCES208H30DavBHisA to obtain *C. glutamicum* AVA-2. Flask cultivation thereafter confirmed a decline in metabolic flux toward GTA. Thus, the yield of 5-AVA increased to 11.42 g/L, while that of GTA decreased to 0.39 g/L. Therefore, blocking the 5-AVA degradation pathway increased 5-AVA production 1.16-fold, while GTA production decreased 4.36-fold relative to the titers obtained for *C. glutamicum* AVA-1 (Fig. S2a). Additionally, batch fermentation using *C. glutamicum* AVA-2 yielded 11.3 g/L of 5-AVA, while GTA accumulation was minimal at 0.5 g/L, with the maximum OD600 reaching 68.1 after 24 h (Fig. S2b). In batch fermentation, l-lysine accumulated to 11.6 g/L (Fig. S2b), whereas no detectable l-lysine was observed in flask culture (Fig. S2a). This difference is consistent with the limited oxygen transfer capacity of shake flasks compared to bioreactor cultivation, which supports higher l-lysine biosynthetic activity. Similar observations have been reported in other

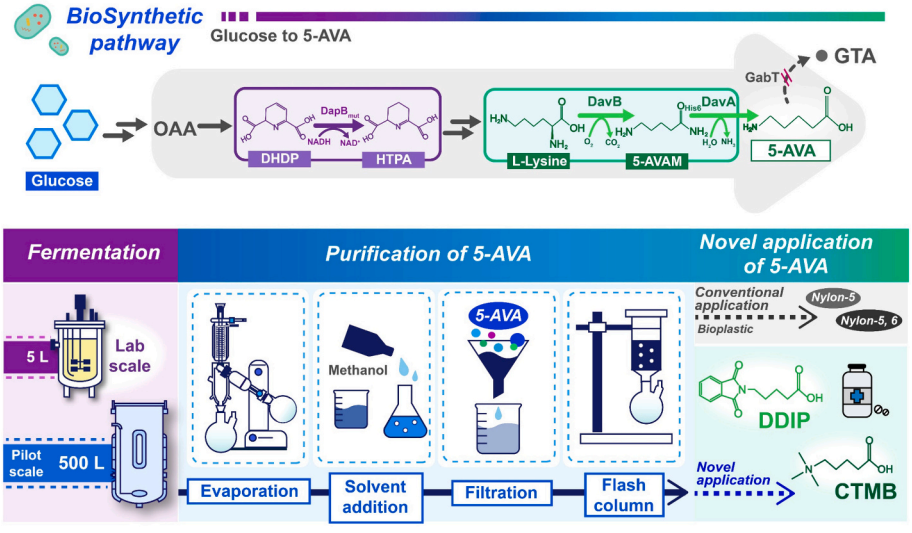

Fig. 1. Schematic overview of a precision fermentation platform for 5-AVA production, purification and novel applications, Glc, glucose; OAA, oxaloacetate; DHDP, *L*-2,3-dihydrodipicolinate; HTPA, 4-hydroxy-tetrahydrodipicolinate; 5-AVAM, 5-aminovleramide; 5-AVA, 5-aminovaleric acid; GTA, glutaric acid; *davA*, δ-aminovaleramidase; *davB*, lysine 2-monooxygenase; *dapB*, dihydrodipicolinate reductase; *gabT*, 4-aminobutyrate aminotransferase, DDIP, 1,3-dihydro-1,3-dioxo-2H-isoindole-2-pentanoic acid; CTMB, 4-carboxy-*N,N,N*-trimethyl-1-butanaminium.

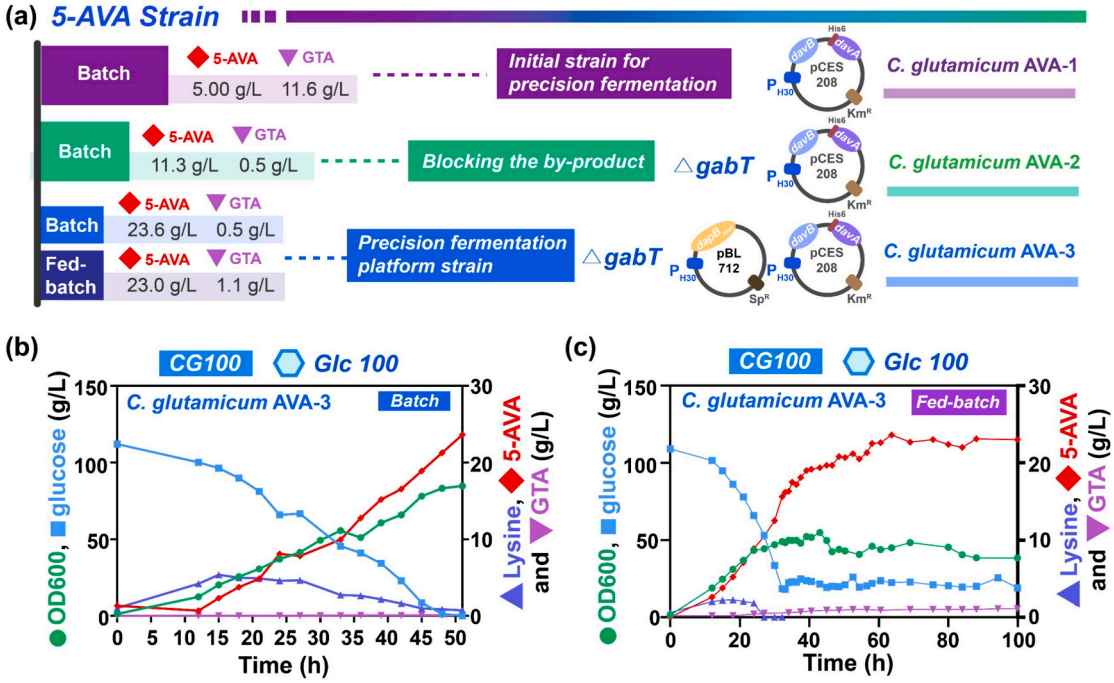

Fig. 2. Establishment of the 5-AVA biosynthetic platform and fermentation results. (a) Construction of the basic 5-AVA biosynthetic platform using *C. glutamicum* AVA-3 and (b) fed-batch fermentation using *C. glutamicum* AVA-3.

production systems [23,24], where secreted l-lysine was not re-incorporated into the intracellular conversion pathway, indicating that once secreted, l-lysine is not efficiently re-uptaken and instead accumulates extracellularly [25]. This combination likely contributes to the leakage of l-lysine into the medium rather than its complete intracellular utilization. Consequently, batch fermentation using *C. glutamicum* AVA-2 under the same conditions as were applied in *C. glutamicum* AVA-1 batch experiments resulted in a 23.2-fold decline in GTA accumulation and a 2.26-fold increase in the 5-AVA titer. These findings indicated that targeted *gabT* deletion effectively suppressed catabolic GTA flux from 5-AVA and improved biosynthetic efficiency.

While *gabT* deletion effectively reduced 5-AVA degradation and improved its titer, further enhancement of 5-AVA productivity is required for industrial application. To improve 5-AVA productivity in this regard, we reinforced the l-lysine biosynthetic pathway, which directly leads to 5-AVA formation. Since l-lysine synthesis from aspartate consumes four moles of NADPH per mole of l-lysine, limited intracellular NADPH availability can restrict l-lysine flux and consequently, 5-AVA production [26]. Therefore, based on the results of our previous study on GTA biosynthesis, we reasoned that 5-AVA production could be further enhanced by introducing the native NADPH-dependent DapB with an NADH-utilizing mutant ($DapB_{mut}$, C115G, G116C). Thus, the precision fermentation platform strain, *C. glutamicum* AVA-3, harboring both pCES208H30DavBHisA and pBL712H30$DapB_{mut}$, was developed. After the colony selection (Fig. S3), in batch fermentation under the same conditions as were applied for *C. glutamicum* AVA-1 and *C. glutamicum* AVA-2, its 5-AVA titer was 23.6 g/L, while GTA accumulation was minimal at 0.5 g/L, and the maximum OD600 observed after 51 h was 84.8 (Fig. 2b). This 5-AVA yield represented a 4.72-fold increase relative to that obtained using *C. glutamicum* AVA-1. Subsequently, in fed-batch fermentation using *C. glutamicum* AVA-3, the 5-AVA titer was 23.0 g/L with the maximum OD600 at 54.9 and GTA accumulation at 1.1 g/L. Further extending the duration of cultivation to 100 h did not result in any significant increase in the 5-AVA titer (23.0 g/L), suggesting the need for further process improvement (Fig. 2c).

3.2. Development of tailored media suitable for precision fermentation to improve 5-AVA production

Despite our metabolic engineering efforts as highlighted in Section 3.1, 5-AVA yield remained suboptimal, with the maximum yields in both batch and fed-batch fermentations at 23.6 and 23.0 g/L, respectively, and extending the cultivation time to 100 h offered minimal improvements. Although product inhibition was initially considered a limiting factor, previous studies have shown that *C. glutamicum* growth is only reduced by approximately 50 % at 70 g/L of 5-AVA [12], indicating that product toxicity is unlikely to be the primary constraint. Instead, these findings suggest that the current culture conditions may not fully support the metabolic potential of the engineered strain (e.g., *C. glutamicum* AVA-3).

Therefore, we developed cultivation media tailored for precision fermentation to improve 5-AVA production, specifically evaluating the effect of medium composition on 5-AVA yield, as summarized in Table 2. The corresponding titers are listed in Table 3. Given that 5-AVA is a growth-associated metabolite, our initial efforts were aimed at identifying medium additives that could promote biomass accumulation [27,28]. Among these, polypeptone, a nitrogen-rich supplement abundant in amino acids and peptides, has emerged as a promising additive for enhancing microbial metabolic activity and growth rate [29]. To assess its effect on *C. glutamicum* growth and 5-AVA production, batch fermentations using *C. glutamicum* AVA-3 were conducted in CG100 medium supplemented with 15 g/L polypeptone (designated CG100P). After 40 h of cultivation, the strain achieved a maximum OD600 of 87.2, producing 20.5 g/L of 5-AVA, with l-lysine and GTA at 2.5 and 0.6 g/L, respectively (Fig. 3a). In comparison, control fermentations in CG100 medium without polypeptone reached an OD600 of 84.8 and yielded 23.6 g/L of 5-AVA after 54 h. Despite the comparable 5-AVA titers, polypeptone supplementation (CG100P) resulted in a 1.02-fold increase in biomass, highlighting its positive effect on cell growth. It also resulted in a 1.35-fold decrease in cultivation time, from 54 h to 40 h. These findings demonstrated that polypeptone supplementation enhanced cellular metabolic activity, improved process kinetics, and was particularly beneficial for scaling up 5-AVA production in fed-batch systems.

Table 2
Improved composition of the medium used in this study.

Medium name	Concentration of components (per liter)	Purpose of use
CG50	15 g of yeast extract, 15 g of $(NH_4)_2SO_4$, 50 g of glucose, 2.0 g of KH_2PO_4, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $FeSO_4{\cdot}7H_2O$, 0.01 g of $MnSO_4{\cdot}H_2O$, and 15 g of $CaCO_3$	Pre-cultivation
CG100	15 g of yeast extract, 15 g of $(NH_4)_2SO_4$, 100 g of glucose, 2.0 g of KH_2PO_4, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $FeSO_4{\cdot}7H_2O$, and 0.01 g of $MnSO_4{\cdot}H_2O$	Main cultivation
CG100P	15 g of yeast extract, 15 g of $(NH_4)_2SO_4$, 100 g of glucose, 2.0 g of KH_2PO_4, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $FeSO_4{\cdot}7H_2O$, 0.01 g of $MnSO_4{\cdot}H_2O$ and 15 g of polypeptone	Main cultivation: To enhance microbial metabolic activity
CG100PM	15 g of yeast extract, 15 g of $(NH_4)_2SO_4$, 100 g of glucose, 2.0 g of KH_2PO_4, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $FeSO_4{\cdot}7H_2O$, 0.01 g of $MnSO_4{\cdot}H_2O$, 15 g of polypeptone, 1.3 mg of $(NH_4)_2MoO_4$, 5 mg of $CuSO_4$, 10 mg of $ZnSO_4$, and 5 mg of $NiCl_2$	Main cultivation: affects intracellular metabolism and enzyme activity

These findings further underscored the value of precision fermentation strategies in improving production efficiency. Subsequently, to further enhance 5-AVA production, we supplemented CG100P with trace metal salts, such as $(NH_4)_2MoO_4$ (1.3 mg/L), $CuSO_4$ (5 mg/L), $ZnSO_4$ (10 mg/L), and $NiCl_2$ (5 mg/L), generating the CG100PM medium. Reportedly, these trace elements modulate intracellular metabolic fluxes and enzymatic activities, and thus, have the potential to enhance 5-AVA biosynthesis [30–32]. The 5-AVA titer of batch fermentation using *C. glutamicum* AVA-3 in CG100PM was 26.1 g/L after 40 h, with the highest OD600 reaching 90.38. While l-lysine accumulation increased to 6.90 g/L and GTA accumulation was not detected (Fig. 3b). Therefore, in addition to increasing 5-AVA production 1.11-fold relative to CG100, CG100PM increased biomass (OD600) by 1.07-fold. Moreover, the fermentation profile in CG100PM exhibited greater stability with respect to both cell growth and 5-AVA production, indicating that this medium is suitable for the metabolically engineered strain in precision fermentation. This enhanced performance supports the applicability of this medium in fed-batch systems and highlights its potential in improving production efficiency and process robustness.

3.3. Demonstration of the scalability of 5-AVA production via precision fermentation

To validate the suitability and scalability of the optimized medium and cultivation conditions in 5-AVA production, we performed *C. glutamicum* AVA-3 fed-batch fermentation experiments using 5 L lab-scale and 500-L pilot-scale systems. Under cultivation at 30 °C, 600 rpm, and 5 vvm, the 5-AVA titer of the 5 L lab-scale system after 109 h was 51.8 g/L, which to the best of our knowledge, is the highest reported 5-AVA titer to date (Table 4). Further, at the end of the fermentation process, l-lysine and GTA concentrations were generally minimal at 1.09 and 3.3 g/L, respectively, with the maximum OD600 of the system reaching 55.4 (Fig. 4a). Relative to the *C. glutamicum* AVA-3-only system, employing the improved medium supplemented with polypeptone and trace metals and *C. glutamicum* AVA-3 led to a 2.25-fold increase in 5-AVA production under identical fermentation conditions. These findings indicated that the final strain-medium system established in this study is highly improved for efficient 5-AVA biosynthesis. Additionally, relative to the previously reported maximum 5-AVA titer of 46.5 g/L obtained using an l-lysine-overproducing recombinant strain engineered to express DavBA and GABA III permease (PP2911) under the Ptuf promoter, along with *lysE* (lysine exporter), *gabTDP* (γ-aminobutyrate catabolic operon), and *argD* (*N*-acetylornithine transaminase) deletions [12], our system showed a 1.11-fold improvement in production titer. These findings specifically highlight the industrial-scale potential of our optimized *C. glutamicum* AVA-3 strain.

To evaluate the industrial applicability of the optimized process, we scaled up the fed-batch fermentation process to a 500 L fed-batch fermentation system. After cultivation for 140 h using CG100PM, a final 5-AVA titer of 44.4 g/L was obtained, and this high 5-AVA titer was accompanied with minimal l-lysine production at 1.18 g/L and no detectable GTA accumulation (Fig. 4b). Additionally, relative to the fed-batch fermentation in the CG100 medium using *C. glutamicum* AVA-3, the optimized system achieved a 1.93-fold increase in 5-AVA titer under equivalent fermentation conditions. It also exhibited only a 14.3 % decline in productivity relative to the 5 L system, indicating minimal performance loss upon up-scaling. Additionally, considering that up-scaling often involves complex variables, such as oxygen transfer, thermal regulation, mixing, and agitation, which do not inherently guarantee increased product titers [33], these findings highlight the robustness and industrial applicability of the improved cultivation conditions and further support the feasibility of the developed system as a precision fermentation platform for scalable 5-AVA production.

Table 3
Comparison of 5-AVA titer according to medium modification conducted in this study.

Strain	Medium name	Scale / working volume (L)	Fermentation type	Total fermentation time (h)	Highest OD_{600nm} during fermentation	Product (g/L)			$Y_{p/s}$	Productivity (g/L/h)
						5-AVA	L-Lysine	GTA		
AVA-1	CG100	5 / 1	Batch	24	70.0	5.0	1.0	11.6	0.11	0.21
AVA-2	CG100	5 / 1	Batch	24	69.7	11.3	11.6	0.5	0.11	0.47
AVA-3	CG100	5 / 1	Batch	51	84.8	23.6	0.74	0.5	0.21	0.46
AVA-3	CG100	5 / 1	Fed-batch	100	54.9	23.0	*	1.1	0.08	0.23
AVA-3	CG100P	5 / 1	Batch	40	87.2	20.5	2.50	0.6	0.20	0.51
AVA-3	CG100PM	5 / 1	Batch	40	90.38	26.1	6.90	*	0.20	0.65
AVA-3	CG100PM	5 / 1	Fed-batch	109	55.4	51.8	1.10	3.6	0.20	0.48
AVA-3	CG100PM	500 / 200	Fed-batch	140	156	44.4	1.20	*	0.10	0.32

* Not detected.

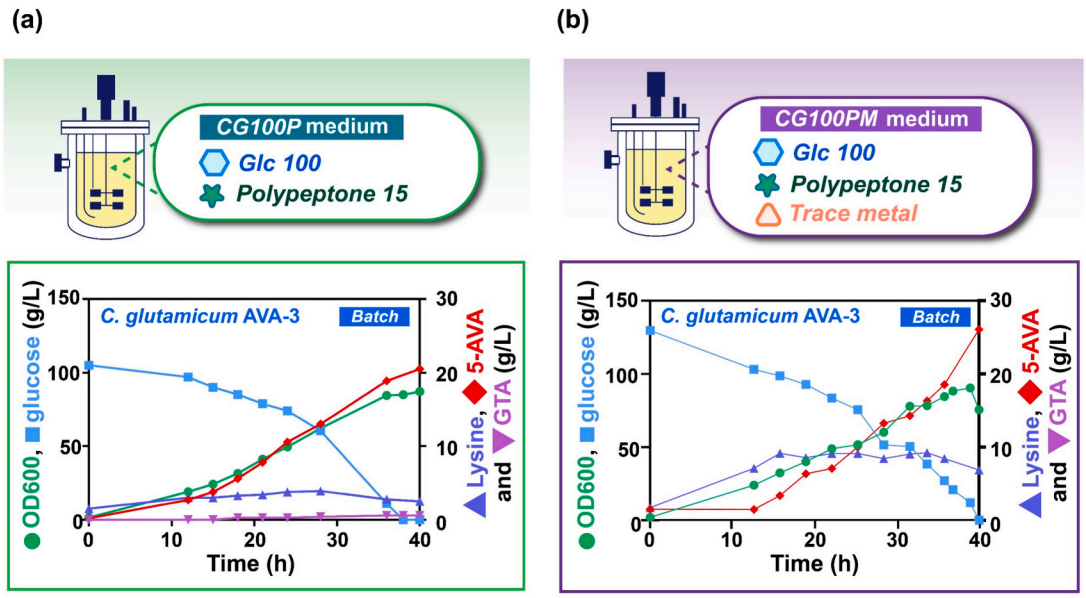

Fig. 3. Effect of tailored media formulation on 5-AVA production in precision fermentation. (a) Fermentation results using the CG100P medium with polypeptone supplementation and (b) fermentation results using CG100PM medium with trace metal supplementation.

Table 4
Summary of reported results in previous studies with this study from glucose-based fed-batch fermentation.

Strain	Scale / working volume (L)	Medium	5-AVA (g/L)	Yield (g/g)	Productivity (g/L/h)	Reference
C. glutamicum LYS-12	1 / 0.3	160 g of glucose, 2 g of $MgSO_4$, 2 g of K_2HPO_4, 2 g of KH_2PO_4, 2 g of urea, 40 g of $(NH_4)_2SO_4$, 20 g of yeast extract and additional medium components 1*	46.5	0.34	0.61	[12]
C. glutamicum BE (KCTC 12390BP)	5 / 2	80 g of glucose, 25 g of $(NH_4)_2SO_4$, 15 g of yeast extract, 2 g of citrate, 1.25 g of KH_2PO_4, 1.25 g of Na_2HPO_4 and additional medium components 2**	33.1	0.10	0.22	[10]
C. glutamicum KCTC1857	2.5 / 0.5	100 g of glucose, 30 g of yeast extract, 30 g of $(NH_4)_2SO_4{\cdot}7H_2O$, 0.5 g of KH_2PO_4 and additional medium components 3***	39.93	0.11	0.54	[11]

* Additional medium components 1: 50 mg of $CaCl_2$, 50 μg of biotin, 20 mg of β-alanine, 20 mg of thiamine HCl, 20 mg of nicotinic acid, 1.3 mg of $(NH_4)_6Mo_7O_{24}$, 10 mg of $FeSO_4$, 10 mg of $MnSO_4$, 5 mg of $CuSO_4$, 10 mg of $ZnSO_4$, 5 mg of $NiCl_2$, and 1 mL of antifoam reagent.

** Additional medium components 2: 1.25 g of $MgSO_4{\cdot}7H_2O$, 165 mg of $CaSO_4{\cdot}2H_2O$, 70 mg of $FeSO_4{\cdot}7H_2O$, 30 mg of $ZnSO_4{\cdot}7H_2O$, 9 mg of $MnSO_4{\cdot}H_2O$, 650 μg of $CoSO_4{\cdot}5H_2O$, 600 μg of $CoSO_4{\cdot}7H_2O$, 480 μg of $NiSO_4{\cdot}6H_2O$, 400 μg of boric acid, 85 μg of $Na_2MoO_4{\cdot}2H_2O$, 30 mg of calcium pantothenate, 9 mg of nicotine amide, 7.5 mg of thiamine·HCl, 3 mg of biotin, and 1 mL of antifoam.

*** Additional medium components 3: 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $MnSO_4{\cdot}H_2O$, 0.01 g of $FeSO_4{\cdot}7H_2O$, 0.5 mg of biotin, and 0.3 mg of thiamine·HCl.

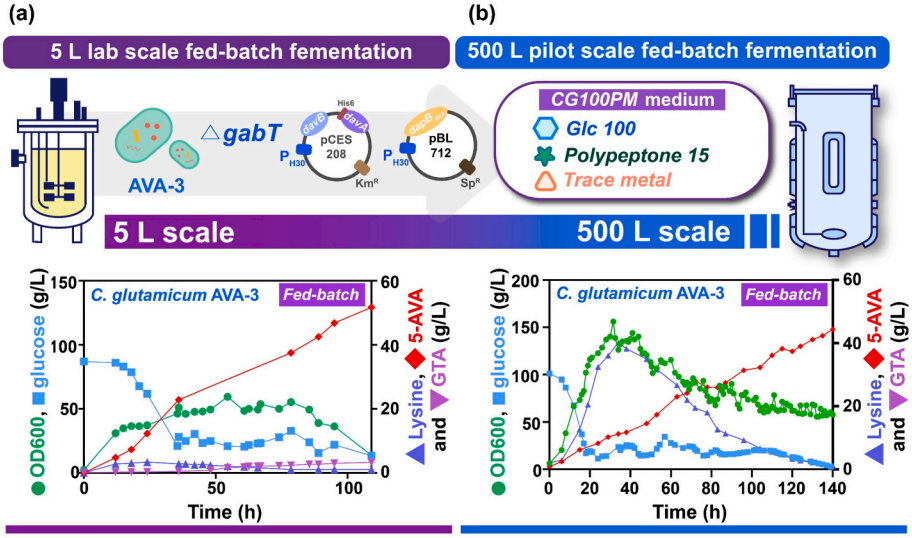

Fig. 4. Scale-up of 5-AVA production after culture condition optimization. (a) Fed-batch fermentation at a 5 L scale after optimization, (b) scale-up procedure from lab-scale to pilot scale and (c) 5-AVA production at 500 L pilot scale. 5-AVA, 5-aminovaleric acid.

3.4. Establishing a scalable downstream process for high-purity 5-AVA production

To effectively utilize biogenic 5-AVA, we initially conducted separation and purification procedures aimed at significantly enhancing its purity (Fig. 1). Given its presence in an aqueous solution containing various salts and other impurities, the first goal in this regard was to remove byproducts and excess salts. Even though a few studies have reported the purification of 5-AVA using adsorbents and column chromatography [13,34], in this study we adopted an organic solvent-based precipitation approach, which presents as a more practical and scalable approach with a high potential for industrial application. Initially, the aqueous solution containing 5-AVA at 37.1 g/L was concentrated via evaporation under reduced pressure. Thereafter, methanol was added to induce salt precipitation. Thus, large quantities of solid salts were precipitated and removed via filtration. ^{1}H NMR spectroscopy analysis confirmed that the filtered solids contained almost no organic compounds (Fig. S4), implying that 5-AVA remained entirely in the filtrate (i.e., ~99 % recovery from the initial solution). The filtrate was further evaporated under reduced pressure to remove residual methanol and water. Following this step, ethanol was added to the concentrated residue to precipitate more solid impurities, which were also removed via filtration. These sequential steps effectively reduced the salt content of the 5-AVA solution, facilitating subsequent purification and preventing the formation of a viscous residue after evaporation. The resulting filtrate was preliminarily purified via flash column chromatography using methanol and dichloromethane. Thus, the yield of 5-AVA was 12.1 g, representing a recovery rate of ~65 %. Finally, high-purity 5-AVA was obtained as a yellow powder via precipitation, with the recovery rate being ~50 % from a mixed solvent system of methanol and dichloromethane. These observations were further confirmed via ^{1}H NMR, ^{13}C NMR, and ESI-MS spectroscopy (Fig. S5-S7). We believe that the solvent-based recovery approach developed in this study provides valuable insights for building robust and scalable downstream strategies for biogenic 5-AVA purification, and unlike adsorptive or membrane-based techniques, this process relies on well-established unit operations, including evaporation, solvent-induced precipitation, and filtration, which can be readily implemented and scaled using existing industrial infrastructure. Additionally, the mild processing conditions employed in this study not only preserved product integrity but also minimized the formation of degradation byproducts. This efficient solvent recovery method also enhanced both the economic and environmental sustainability of the 5-AVA production process. Taken together, these findings underscored the practical potential of solvent-mediated purification as a viable platform for the industrial production of high-purity bio-based 5-AVA [35]. These purification results, which will be further validated at larger scales, highlight the potential of 5-AVA to serve as a sustainable alternative to commercial platform chemicals. A techno-economic assessment provided in the Supplementary Material (Fig. S8, S9; Tables S2, S3) further supports this finding, suggesting that the proposed strategy is cost-competitive with commercial 6-aminocaproic acid.

3.5. Chemical valorization of bio-based 5-AVA into value-added compounds for novel applications

To demonstrate the practical applicability and molecular versatility of biogenic 5-AVA, we used it as starting material to synthesize two representative organic derivatives as a proof-of-concept. Generally, 5-AVA is often used as the starting material owing to its unique structure, comprising a linear C5 backbone, featuring both amino and carboxyl functional groups, which render it an excellent platform molecule for diverse chemical transformations. Additionally, relative to its conventional petroleum-derived counterparts, biogenic 5-AVA presents as a sustainable and low-carbon alternative, aligning with current efforts in green chemistry and a circular bioeconomy.

1,3-Dihydro-1,3-dioxo-2H-isoindole-2-pentanoic acid (DDIP, compound 1) serves as a promising synthetic precursor for the development of antibody-drug conjugate (ADC) linkers, which are critical components in targeted cancer therapy [36]. The presence of a reactive phthalimide moiety and a carboxylic acid side chain in this compound provide structural versatility for further functionalization and bioconjugation. 4-Carboxy-*N,N,N*-trimethyl-1-butanaminium (CTMB, compound 2), also referred to as 5-AVA betaine, is a quaternary ammonium derivative that can be employed as a molecular probe or metabolic tracer in biological and biomedical research, particularly in studies related to amino acid transport, neurotransmitter analogs, and microbial metabolism [37].

DDIP (compound 1) was synthesized via dehydrative condensation between 5-AVA (115 mg, 0.982 mmol) and phthalic anhydride (145 mg, 1 equiv.). Owing to the limited solubility of 5-AVA in organic solvents, a dual-solvent system, comprising toluene and methanol, was employed to enhance solubility and reaction efficiency (Fig. 5a). After purification, this compound was successfully isolated (163 mg, 67.3 %) and its structure was confirmed via spectroscopic analyses (Fig. 5c and Fig. S10–S12). Alternatively, CTMB (compound 2) was synthesized via the methylation of 5-AVA (117 mg, 1.0 mmol) using iodomethane (311 μL, 5 equiv.) and potassium bicarbonate (400 mg, 4 equiv.) in methanol followed by hydrolysis using conc. HCl to yield the final product (125 mg, 78 %) (Fig. 5b). NMR and mass spectrometry were then employed to confirm successful synthesis (Fig. 5d and Fig. S13–S15).

Overall, this study highlighted the potential of biogenic 5-AVA as a versatile and sustainable building block for the synthesis of high-value functional materials. Specifically, the successful synthesis of DDIP (compound 1; an ADC linker precursor) and CTMB (compound 2; a quaternary ammonium derivative, respectively) demonstrated the practical utility and adaptability of the biogenic 5-AVA in real-world applications. Additionally, its unique molecular structure, featuring both amino and carboxyl groups, enabled diverse chemical transformations into derivatives with broad applicability in biopolymer production [38], cancer therapy [39], and advanced material production [40].

4. Conclusion

In this study, a precision fermentation platform for high-titer 5-AVA production using *C. glutamicum* AVA-3 was established. Its lab-scale and pilot-scale application yielded the highest reported titers, i. e., 51.8 and 44.4 g/L, respectively, to date. Further, we developed a scalable downstream purification strategy that allowed the efficient, and sustainable recovery of high-purity 5-AVA (approx. 50 %). These findings highlight the potential of precision fermentation and our purification method in supporting the scalable production of value-added biochemicals. Simultaneously, our findings demonstrated the potential of bio-based 5-AVA to be valorized into high-value compounds, such as a phthalimide derivative for ADC linker applications and 5-AVA betaine as a promising postbiotic candidate. Further efforts aimed at enhancing 5-AVA production by engineering metabolic flux toward its generation using metabolic engineering and synthetic biology tools remain necessary. Overall, the developed platform demonstrated robustness in tailoring product profiles for application-specific material and health-related uses. Thus, it presents as a versatile and robust model for precision fermentation–driven biomanufacturing that has the potential to contribute to the establishment of a bioeconomy.

CRediT authorship contribution statement

Yunhee Jeong: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Younghoon Kim:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Kyung-An Kim:** Writing – original draft, Validation, Methodology, Investigation, Data curation.

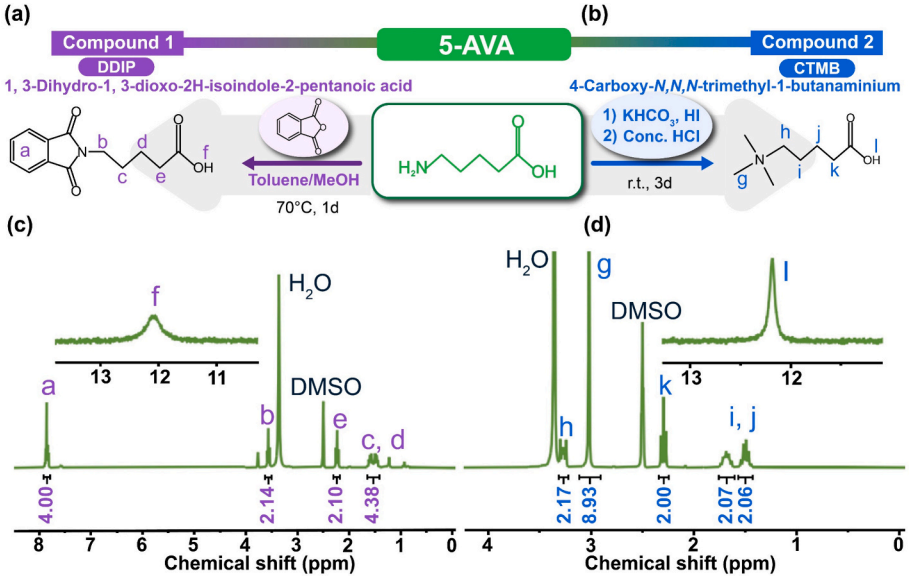

Fig. 5. Synthesis routes (a for DDIP, b for CTMB) and corresponding ^{1}H NMR spectra (c for DDIP, d for CTMB) of 1,3-dihydro-1,3-dioxo-2H-isoindole-2-pentanoic acid (DDIP, compound 1) and 4-carboxy-*N*,*N*,*N*-trimethyl-1-butanaminium (CTMB, compound 2), synthesized from 5-AVA via respective chemical valorization pathways.

Yu Jung Sohn: Writing – original draft, Validation, Methodology, Investigation, Data curation. **KwangYoung Park:** Validation, Methodology, Investigation, Data curation. **Eunchae Song:** Validation, Methodology, Data curation. **Kyungmoon Park:** Validation, Data curation. **See-Hyoung Park:** Validation, Data curation. **Yung-Hun Yang:** Validation, Data curation. **Jong Hyun Choi:** Validation, Data curation. **Seojin Shin:** Formal analysis, Software. **Wangyun Won:** Software, Validation. **Si Jae Park:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Hyun Gil Cha:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Hee Taek Kim:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2025.168950.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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