Research Article

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Comparative routes to 7-carboxymethyl-pterin: A useful medicinal chemistry building block

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Abstract: Pterins, such as folate and bioppterin, and their derivatives hold significant importance given their biological relevance, as well as the numerous pterin-based inhibitors developed for targeting various biological targets. For this reason, pterins can be viewed as a *privileged scaffold*, as the discovery of new pterin analogs gives rise to a vast array of potential drug candidates. 7-carboxymethyl-pterin (7-CMP) represents a useful scaffold for the rapid generation of structurally diverse pterin amides and has been a key building block in medicinal chemistry. In an effort to facilitate rapid generation of this pterin scaffold, we have explored multiple routes towards 7-CMP to assess the most efficient method of generation. Methods were evaluated based on yield, regioselectivity, reaction time, and hazardous reaction conditions. This work can aid in the synthesis and discovery of new pterin-based drug candidates.

Keywords: pterin, regioselective synthesis, medicinal scaffold

1 Introduction

The importance of pterin derivatives stems from discovery of numerous biologically relevant pterins, such as folate and bioppterin and their tetrahydro-derivatives [1,2]. These pterins play key roles in cell division, degradation of nucleic acids and amino acids, neurotransmitter biosynthesis, and nitric oxide production [1,3–7]. Due to this biological relevance, several pterin-based inhibitors have been developed for targets such as ricin [8,9], methionine synthase [10], leishmaniasis [11], nitric

oxide synthase [12,13], as well as the anti-folate drug methotrexate. In this regard pterins can be viewed as a *privileged scaffold*, as the discovery of new pterin chemistry and pterin analogs gives rise to a vast array of potentially pharmaceutically relevant compounds. Additionally, the donor/acceptor hydrogen-bonding array has made deazaguanine derivatives useful in a number of supramolecular assemblies [14]. Any method that provides a pterin with a readily manipulated functional group should thus be of utility to medicinal and supramolecular chemists alike who seek a rapid pathway to their desired heterocycle.

We have been particularly interested in pterins substituted at the 7-position, as these have shown promise in the development of inhibitors of ricin, shigella, and aldose reductase [8,9,15,16]. Regioselective synthesis of pterins has been reported, but can be complicated by the notorious insolubility of pterins in most solvents [17]. One reason for this insolubility is the complimentary hydrogen bond donors and acceptors throughout the molecule, which make for strong solute-solute interactions and self-assembly. This can be dealt with by preemptive modifications to the pteridine which disrupt these interactions [18–20]. The installation and removal of these groups affect the overall yield and time necessary to generate new pterins. Taylor et al. developed a very elegant method for synthesizing isomerically pure pterins, although it does require a somewhat lengthy multi-step approach [21]. A straightforward and regioselective access to a pterin containing an easily manipulated functional handle should be of great utility to those seeking to generate libraries of diverse pterin derivatives. A key pterin building block of interest is 7-carboxymethyl-pterin (7-CMP; **1**) (Figure 1). The ester functional group allows for conversion to numerous other derivatives, most commonly amides, which have shown activity against various biological targets [9,16,22–24]. Most importantly, compound **1** was used in a key DBU-amidation reaction, which allows access to numerous structurally diverse pterin amides using mild reaction conditions, often in 5–10 min [25]. In this way, compound **1** should be viewed as an essential building block for pteridine chemists.

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Figure 1: 7-carboxymethyl-pterin (**1**).

Given the importance of compound **1** highlighted above, we have explored a variety of synthetic methods to access this important molecule. Four main pathways have been explored, referred to as Paths A–D, and the viability of these methods have been compared with respect to their overall yield, ease of reaction conditions, regioselectivity, and overall time necessary to complete the synthesis. These comparisons should be of great use to those wishing to access compound **1** for future drug development and beyond.

2 Results and discussion

All envisioned routes to compound **1** used 2,5,6-triamino-4-pyrimidone (**4**) as a common intermediate, so we began with the synthesis of this key intermediate (Scheme 1). 2,6-Diamino-4-pyrimidone (**2**) was produced through reaction of ethyl cyanoacetate with guanidinium chloride in ethanolic sodium ethoxide, and this compound was then nitrosylated through use of sodium nitrite in acetic acid to provide **3**. The reduction of **3** to **4** can be accomplished either through the use of sodium dithionite, or with 40% (w/w) ammonium sulfide. When using commercial sodium dithionite, we found that the reduction did not complete, even with extended reaction time. However, when sodium dithionite was freshly prepared from sodium sulfite and zinc dust, the reduction was complete in roughly 1 h with a yield of 89%. We also explored the reduction using 40% (w/w) ammonium sulfide, which was successful but gave a slightly lower yield of 84% and required a longer reaction time. Given the generally foul odor associated with the use of ammonium sulfide, and the lower yield, sodium dithionite reduction was seen as the superior method. Through the methods described above, compound **4** was generated with an overall yield of 65% over 3 steps.

Scheme 1: Synthesis of 2,5,6-diamino-4-pyrimidone (**4**).

With the necessary pyrimidine scaffold in hand, we then explored a variety of routes toward **1**, as shown in Scheme 2. To provide a brief overview, “Path A” relies on conversion of **4** to 7-methylpterin (**5**) which can then be oxidized to the corresponding carboxylic acid (**6**), thereby giving **1** via Fischer-esterification [26]. Alternatively, both “Paths B and C” rely on a Minisci-type acyl radical reaction with an unsubstituted pterin (**7**) to provide **1** directly [27–29]. “Path D” would make use of a reported susceptibility of the pterin N-oxide (**8**) toward nucleophilic addition at the 7-position, and subsequent oxidative cleavage to again provide the carboxylic acid **6** [30]. For each pathway explored, attempts were made to optimize each step, as discussed below.

The first route toward **1**, “Path A”, required condensation of **4** with methylglyoxal. A number of reports have been made on ways to control the regioselectivity of this step, as it can also produce the isomeric 6-methylpterin [26,31,32]. Typically, in acidic conditions and/or in the presence of reagents which reversibly mask the reactivity of the aldehyde (bisulfite or thiols), the 6-methyl isomer dominates, while in basic conditions, the 7-methyl isomer is the major product. Routinely, a mixture of regioisomers is produced and separation requires acylation of the exocyclic nitrogen, recrystallization, and subsequent removal of the acyl group [32]. When **4** was suspended in 70°C water followed by addition of methylglyoxal, roughly 9:1 ratio of the 7-methyl and 6-methyl pterin isomers were recovered. Rerunning the reaction in the presence of sodium bicarbonate, and increasing the temperature to 100°C provided **5** almost exclusively, with less than 2% of the undesired isomer detected. Fortuitously, in the subsequent permanganate oxidation, only the desired 7-carboxypterin (**6**) was isolated. The final step of “Path A” required overnight reflux of **6** in methanol and sulfuric acid. Conversion was slow, mostly owing to the poor solubility of **6**. Path A provided **1** with 50% overall yield from **4** over three steps.

Given that the pterins are π -electron deficient, they cannot undergo traditional Friedel-Crafts acylation. However, they do readily react with acyl radicals via the Minisci reaction, and as reported previously, this reaction is regiospecific for acylation at the 7-position [29]. This is

Scheme 2: Four different synthetic paths to **1**.

one of the most attractive attributes of this route, as pterin **7** can be easily prepared with glyoxal without worrying about regioselectivity in the condensation. The original conditions for the Minisci step involved adding 2 equivalents of iron sulfate, 4 equivalents of methyl pyruvate, and 2.5 equivalents of hydrogen peroxide to a solution of **7** in 2.5 M sulfuric acid. These conditions initially provided **1** with a yield of 41%. While this yield was low, this route benefits from the reaction occurring under 10 min and in the ease of product isolation. Initially pterin is dissolved only due to the high acidity of the reaction, and because the product is less basic it allows the product to selectively precipitate out. Given this selective product precipitation, it may seem a reasonable way to improve the yield would be to partially neutralize the reaction to further induce precipitation. Unfortunately, treatment of the reaction filtrate with base also results in formation of rust-like iron hydroxides. In an effort to minimize this issue, we explored varying the amount of iron sulfate used (Table 1). Given that Fe(II) is catalytic in the Minisci mechanism, we were surprised to find a negative effect on the yield when using less than one equivalent. Nevertheless, maintaining a single equivalent of iron sulfate provided the most ideal yield (60%) given it allowed for some flexibility in adjusting the pH before the

iron hydroxide co-precipitation. In this way, “Path B” provided **1** with 57% overall yield from **4** over 2 steps.

With the primary downside to “Path B” stemming from the iron salt, we next sought to explore an alternative set of Minisci conditions, whereby the acyl radical is generated by a decarboxylative step in the presence of persulfate and Ag(I) [33]. This route, “Path C,” was initially seen as the superior method, as the silver ion could remain dissolved if basification was carried out with ammonia and thereby allowing more flexibility in adjusting the pH to allow for product precipitation. The potassium salt of monomethyl oxalate was first generated via a controlled hydrolysis of dimethyl oxalate [34]. While treatment of **7** with this ester source followed by sodium persulfate and silver nitrate did provide **1**, these alternative Minisci conditions only resulted in 14% yield. Numerous efforts were made to optimize this

Table 1: Effect of varying Fe(II) on reaction yield

Entry	FeSO ₄ equivalents	Yield (%)
1	2.0	41
2	1.5	48
3	1.0	60
4	0.5	33

result, through varying equivalents of all reactants and through changing the persulfate salt used (sodium, potassium, and ammonium persulfate were explored), however we were unable to improve the yield for this step. Path C was thus deemed unsuitable, with a 13% overall yield from **4** over 2 steps.

The final path envisioned for this synthesis came from the work of Taylor and Jacobi, where they showed under appropriate conditions that the pterin N-oxide (**8**) could be attacked by active methylene nucleophiles [30]. In their work, they treated **8** in hexamethylphosphoric acid triamide and acetic anhydride with ethyl cyanoacetate to provide a mixture of products with a new carbon-carbon bond forming at the 7-position of the pterin ring (Figure 2). The authors confirmed the regiochemistry of this reaction by permanganate oxidation of the mixture to provide 7-carboxy-pterin (**6**).

Given the chemistry described above can be used to regioselectively provide the direct precursor to **1**, we explored this as “Path D.” Sadly, in our hands we only recovered trace amounts of **6**, despite numerous attempts. This is mostly consistent with the previous authors’ work, as they reported roughly 30% yield for the initial nucleophilic attack of the N-oxide, followed by roughly 2% yield for the permanganate oxidation step. As such, “Path D” was abandoned as a non-viable route towards **1**.

A general overview and comparison between the four routes, is provided in Table 2. While no route was without its drawbacks, it was concluded that “Path B” represented the most ideal for synthesis of the desired 7-CMP (**1**). Given the utility of **1** as a building block for rapid generation of new functionally diverse pterin analogs, future pteridine chemists would benefit from this synthetic path when building a library of new pterins.

3 Materials and methods

All reagents used were of commercial quality and obtained from Aldrich Chemical Co. and used as received. Proton nuclear magnetic resonance (^1H NMR) and carbon (^{13}C NMR) spectra were recorded in deuterated dimethylsulfoxide ($\text{DMSO}-d_6$) or in deuterium oxide/potassium deuterioxide ($\text{D}_2\text{O}/\text{KOD}$) with a Bruker spectrometer using the solvent as the reference. Peak splitting patterns for ^1H NMR are reported

as singlets (s), doublets (d), or broad (br) where appropriate. Ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS) was performed on a Waters Acquity UPLC H-class system equipped with a quadrupole diode array (QDa) detector. All filtrations were performed with vacuum. Evaporation of solvents was performed using a Büchi RE 121 rotary evaporator. Solids were dried using a GCA Precision Scientific model 19 vacuum drying oven. Melting points were recorded using a Stanford Research System DigiMelt MPA 160.

3.1 2,6-Diamino-4-pyrimidone (**2**)

This compound was synthesized as reported in the literature [35]. A 500 mL oven dried 3-neck round bottom flask was charged with 200 mL anhydrous ethanol, and this was cooled in an ice bath. Sodium metal (9.6 g; 417 mmol) was added to the ice-cold ethanol with vigorous stirring, with an attached reflux condenser. The solution was stirred until the sodium had fully dissolved and the reaction was allowed to come to room temperature. To this was added 20 mL (188 mmol) of ethyl cyanoacetate, followed by 18 g (188 mmol) guanidinium chloride. The mixture was refluxed for 4 h and then cooled to room temperature. The ethanol was removed on a rotary evaporator under vacuum. The solid mass was then dissolved in 200 mL of hot deionized water, and 24 mL of acetic acid was added slowly over the course of 5 min. This solution was then cooled in an ice bath for 1 h, after which the yellow crystals were isolated by vacuum filtration, washed with water, and dried in a vacuum oven to provide 18.9 g (80%) of compound **2**. MP $>300^\circ\text{C}$. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm) = 9.96 (br, NH), 6.27 (br, NH_2), 6.03 (br, NH_2), and 4.54 (s, CH). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm) = 165.9, 164.0, 155.7, and 76.9. LCMS $[\text{M} + 1]^+ m/z = 127$. These data are consistent with that reported in the literature [35].

3.2 2,6-Diamino-5-nitrosopyrimidinone (**3**)

A suspension of 12.9 g (102 mmol) pyrimidine **2** in 180 mL of deionized water was prepared and heated to 80°C , and to this was added 20 mL of acetic acid. Separately, 7.05 g

Figure 2: Reactivity of pterin N-oxides reported by Taylor and Jacobi.

Table 2: Overall comparison of routes to 1

Paths	Number of steps	Overall yield (%)	Total reaction time (h)*	Benefits	Drawbacks
Path A	3	50	48	Mild conditions	Regioisomers; overnight reactions
Path B	2	57	1.5	Fast; regioselective	Hazardous condition; rust byproducts
Path C	2	13	24	Regioselective; no rust byproducts	Low yield
Path D	4	>1	36	Regioselective	Unacceptable yields

* Total reaction time does not include time for drying intermediates between steps.

(102 mmol) NaNO_2 was dissolved in 25 mL of water. The aqueous NaNO_2 solution was added dropwise over 15 min to the solution of compound 2. The reaction was stirred at 80°C for 1 h, and then cooled in an ice bath for 30 min. The pink solid was collected by vacuum filtration, washed with water and then methanol, and dried in a vacuum oven to provide 14.6 g (92%) of compound 3. MP >300°C. ^{13}C NMR (100 MHz, $\text{D}_2\text{O}/\text{KOD}$) δ (ppm) = 176.6, 169.2, 157.8, and 143.5 (as expected in D_2O , the ^1H NMR of this compound showed no signals). LCMS $[\text{M} + 1]^+ m/z = 156$. These data were in accordance with the literature [36].

3.3 2,5,6-Triaminopyrimidinone (4)

This compound can be synthesized through two different methods. *Method 1:* Roughly 3 M solution of sodium dithionite was freshly prepared by dissolving 50 g sodium bisulfite in 80 mL of water and adding 22 g zinc powder, while cooling in an ice bath. The reaction was stirred in the ice bath for 5 min, followed by stirring at room temperature for 30 min. The $\text{Zn}(\text{OH})_2$ precipitate was filtered off, and the filtrate was used directly in the next step. To this freshly prepared sodium dithionite solution was added a suspension of 14 g (90 mmol) of compound 3 in 200 mL of water, followed by the addition of 10 mL of saturated ammonium hydroxide. The reaction was heated to 100°C and stirred for 1 h, during which time the pink color had faded and only a white solid remained. The solid was collected by vacuum filtration, washed with water and then ethanol, and finally dried in a vacuum oven to provide 11.3 g (89%) of compound 4. *Method 2:* A suspension of 14.6 g (94 mmol) of compound 3 and 160 mL of $(\text{NH}_4)_2\text{S}$ (40% w/w solution) was stirred at 50°C overnight. The reaction was then cooled in an ice bath for 1 h, then the solid was collected by vacuum filtration, rinsed several times with water and then methanol, and dried in vacuum oven to provide 11.2 g (84%) of compound 4. Both methods provided material in agreement to that reported in the literature [36]. MP = 247°C (decomp). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm) = 10.2 (br, NH), 5.70 (br, NH_2), 5.36 (br, NH_2), and 3.05 (br, NH_2). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm) = 158.6, 152.9, 149.7, and 99.5. LCMS $[\text{M} + 1]^+ m/z = 142$.

3.4 7-Methylpterin (5)

This compound was synthesized through a modified version of that reported in the literature [37]. A suspension of 1 g (7.1 mmol) of compound 4 in 120 mL of water was

heated to 100°C. To this was added 1.19 g NaHCO₃ (14.2 mmol) with stirring, followed by 2 g of a 40% (w/w) solution of methylglyoxal (1.5 equiv). The reaction was maintained at 100°C for 1 h, before being cooled to room temperature, and stored in a refrigerator overnight. The precipitate was isolated by vacuum filtration, washed with water and then methanol, and dried in a vacuum oven to provide 1.13 g (90%) of compound **5**. MP >300°C. ¹H NMR (400 MHz, D₂O/KOD) δ (ppm) = 8.49 (s, CH) and 2.56 (s, CH₃). ¹³C NMR (100 MHz, D₂O/KOD) δ (ppm) = 175.8, 166.9, 161.5, 158.5, 141.6, 129.1, and 23.8. LCMS [M + 1]⁺ m/z = 178. Spectral data were consistent with the formation of the desired 7-methyl isomer [37]. Less than 2% of the 6-methyl isomer was detected, as determined by ¹H NMR.

3.5 7-Carboxypterin (6)

This compound was prepared through a modified version of that reported in the literature [26]. A 200 mL solution of 2 M NaOH was heated to 90°C, and to this was added 1.1 g (6.2 mmol) of compound **5** with stirring. After full dissolution of **5**, 3 g (18 mmol) of KMnO₄ was added in portions over a 3 h period. The reaction was stirred at 90°C overnight and then cooled to room temperature. Excess KMnO₄ was quenched by addition of Na₂SO₃, and the precipitated MnO₂ was filtered. This precipitate was then washed with 100 mL of hot 0.5 M NaOH, and this second filtrate was acidified by addition of 6 M HCl. The resulting fine yellow precipitate was collected by vacuum filtration, and dried in a vacuum oven to provide 977 mg (76%) of compound **6**. MP >300°C. ¹H NMR (400 MHz, D₂O/KOD) δ (ppm) = 8.66 (s, CH). ¹³C NMR (100 MHz, D₂O/KOD) δ (ppm) = 172.9, 171.0, 164.5, 156.2, 151.9, 138.0, and 129.7. LCMS [M + 1]⁺ m/z = 208. Spectral data were consistent with that reported in the literature, and showed no evidence of the 6-carboxy isomer [26].

3.6 2-Aminopteridinone (pterin; 7)

The sulfate salt of compound **4** was prepared by stirring **4** in cold 1 M H₂SO₄ for 20 min, then filtering and drying under vacuum. 12.6 g (52.7 mmol) of this sulfate salt was suspended in 1 L of water at room temperature. To this was added 13.3 g (105 mmol) Na₂SO₃ which resulted in a lightly tan-colored homogeneous solution. A 40% (w/w) solution of glyoxal (18 g solution, 126 mmol glyoxal) was added to the reaction, and stirring was continued for 1 h, during which time a thick, peach-colored precipitate

formed. This precipitate was collected by vacuum filtration, washed with water and methanol, and then dried in a vacuum oven to provide 8.17 g (95%) of compound **7**. MP >300°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm) = 11.2 (br, NH), 8.64 (d, CH), 8.36 (d, CH), and 6.92 (br, NH₂). ¹³C NMR (100 MHz, D₂O/KOD) δ (ppm) = 172.9, 164.0, 156.3, 148.2, 138.3, and 129.2. LCMS [M + 1]⁺ m/z = 164. The NMR data were identical to an authentic commercial sample (Sigma Aldrich), and were consistent with that reported in the literature [38].

3.7 2-Amino-4-oxo-pteridine 8-oxide (8)

This compound was prepared through a variation of that reported in the literature [39]. A 1.5 g (9.2 mmol) sample of compound **7** was heated in 8 mL of trifluoroacetic acid, and then the reaction was cooled to room temperature. 1.5 mL of 30% (w/w) H₂O₂ was added dropwise, and the reaction was stirred for 1 h. After addition of a small amount of cold water to the reaction, the bright yellow precipitate was collected by vacuum filtration, washed with water, and isopropanol. Addition of isopropanol to the filtrate resulted in additional yellow precipitate. The combined solids were dried under vacuum to provide 1.64 g (>99%) of compound **8**. MP >300°C. ¹H NMR (400 MHz, D₂O/KOD) δ (ppm) = 8.52 (d, CH) and 8.32 (d, CH). ¹³C NMR (100 MHz, D₂O/KOD) δ (ppm) = 175.0, 166.7, 151.2, 141.4, 139.5, and 135.7. LCMS [M + 1]⁺ m/z = 180. Spectral data matched that reported in the literature [39,40].

3.8 7-CMP; 1

Via Path A: To a suspension of 2.0 g (9.7 mmol) of compound **6** in 600 mL of methanol was added 6 mL of concentrated H₂SO₄. The reaction was refluxed overnight, then cooled to room temperature and the majority of the solvent was removed under vacuum. To the thick slurry that remained was added 200 mL of water, and the solution was brought to pH 7 by dropwise addition of 2 M NaOH. The yellow precipitate was isolated by vacuum filtration and dried in a vacuum oven to provide 1.58 g (74%) of compound **1**. *Via Path B:* To a 70 mL solution of 2.5 M H₂SO₄ was added 1.0 g (6.13 mmol) of compound **7**. This suspension was heated and sonicated until dissolved, and then this solution was cooled in an ice bath. To this solution was added 1.71 g (6.14 mmol) FeSO₄·7H₂O with vigorous stirring. In a separate ice bath, 1.73 g of a 30% (w/w) H₂O₂ solution (15.3 mmol peroxide) was cooled,

and to this was added 2.27 mL (24.5 mmol) of methyl pyruvate. This chilled peroxide/pyruvate mixture was then added dropwise to the stirred solution of compound **7** and FeSO_4 from above. The reaction was maintained at 0°C for 10 min, during which time a fine yellow precipitate separated from the solution. This main crop of solid was isolated by vacuum filtration, and the filtrate was partially neutralized by careful addition of Na_2CO_3 , while maintaining the pH below 6, providing a small amount of additional yellow precipitate which was again isolated by vacuum filtration. Both solids were washed thoroughly with water and methanol, and the combined solids were dried in a vacuum oven to provide 813 mg (60%) of compound **1**. *Via Path C:* To a suspension of 1.5 g (9.2 mmol) of compound **7** in 100 mL of water was added 10 mL of concentrated H_2SO_4 , followed by 8.57 g (36 mmol) $\text{Na}_2\text{S}_2\text{O}_8$, and 1.1 g (6.5 mmol) AgNO_3 , with vigorous stirring. To this mixture was added 6 g (42 mmol) of the potassium salt of methyl monooxalate, prepared by its literature procedure [34]. The reaction was heated to 80°C and stirred overnight. After cooling to room temperature, and addition of aqueous ammonia to reach a pH of 6, a yellow precipitate was collected by vacuum filtration, and dried in a vacuum oven to provide 284 mg of compound **1**. All three samples of compound **1** obtained via paths A–C had identical spectra, and matched that reported in the literature for the 7-substituted ester [29]. MP $>300^\circ\text{C}$. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm) = 11.74 (br, NH), 8.85 (s, CH), 7.09 (br, NH_2), and 3.94 (s, CH_3). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ (ppm) = 163.2, 158.4, 152.5, 147.7, 145.0, 142.3, 132.2, and 53.8. LCMS $[\text{M} + 1]^+$ m/z = 222.

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References

- [1] Shane B. Folate in Health and Disease. 2nd edn. Boca Raton, Florida: CRC Press; 2010.
- [2] Kaufman S. A new cofactor required for the enzymatic conversion of phenylalanine to tyrosine. *J Biol Chem.* 1958;230:931–9.
- [3] Scott JM. Folate and vitamin B12. *P Nutr Soc.* 1999;58:441–8.
- [4] Stover P. Folate in Health and Disease. 2nd edn. Boca Raton, Florida: CRC Press; 2010.
- [5] Woo KS, Chook P, Lolin YI, Sanderson JE, Metreweli C, Celermajer DS. Folic acid improves arterial endothelial function in adults with hyperhomocysteinemia. *J Am Coll Cardiol.* 1999;34:2002–6.
- [6] Thony B, Auerbach G, Blau N. Tetrahydrobiopterin biosynthesis, regeneration and functions. *Biochem J.* 2000;347(Pt 1):1–16.
- [7] Channon KM. Tetrahydrobiopterin: regulator of endothelial nitric oxide synthase in vascular disease. *Trends Cardiovasc Med.* 2004;14:323–7.
- [8] Yan X, Hollis T, Svinth M, Day P, Monzingo AF, Milne GWA, et al. Structure-based identification of a ricin inhibitor. *Journal of Molecular Biology.* 1997;266:1043–9.
- [9] Pruet JM, Jasheway KR, Manzano LA, Bai Y, Anslyn EV, Robertus JD. 7-Substituted pterins provide a new direction for ricin A chain inhibitors. *Eur J Med Chem.* 2011;46:3608–15.
- [10] Zhang Z, Tian C, Zhou S, Wang W, Guo Y, Xia J, et al. Mechanism-based design, synthesis and biological studies of N^5 -substituted tetrahydrofolate analogs as inhibitors of cobalamin-dependent methionine synthase and potential anticancer agents. *Eur J Med Chem.* 2012;58:228–36.
- [11] Tulloch LB, Martini VP, Iulek J, Huggan JK, Lee JH, Gibson CL, et al. Structure-based design of pteridine reductase inhibitors targeting African sleeping sickness and the leishmaniasis. *J Med Chem.* 2010;53:221–9.
- [12] Frohlich LG, Kotsonis P, Traub H, Taghavi-Moghadam S, Al-Masoudi N, Hofmann H, et al. Inhibition of neuronal nitric oxide synthase by 4-amino pteridine derivatives: structure-activity relationship of antagonists of (6R)-5,6,7,8-tetrahydrobiopterin cofactor. *J Med Chem.* 1999;42:4108–21.
- [13] Matter H, Kotsonis P, Klingler O, Strobel H, Frohlich LG, Frey A, et al. Structural requirements for inhibition of the neuronal nitric oxide synthase (NOS-I): 3D-QSAR analysis of 4-oxo- and 4-amino-pteridine-based inhibitors. *J Med Chem.* 2002;45:2923–41.
- [14] Todd EM, Zimmerman SC. Bis-ureidodeazapterin (Bis-DeAP) as a general route to supramolecular star polymers. *Tetrahedron.* 2008;64:8558–70.
- [15] Brenk R, Gerber H-D, Kittendorf JD, Garcia GA, Reuter K, Klebe G. From hit to lead: de novo design based on virtual screening hits of inhibitors of tRNA-guanine transglycosylase, a putative target of shigellosis therapy. *Helv Chim Acta.* 2003;86:1435–52.
- [16] Saito R, Suzuki S, Sasaki K. Pterin-7-carboxamides as a new class of aldose reductase inhibitors. *Bioorg Med Chem Lett.* 2016;26:4870–4.
- [17] Nonogawa M, Arai T, Endo N, Pack SP, Kodaki T, Makino K. Hydrogen bond removal of pterin derivative whose structure is similar to nucleic acid bases. *Nucleic Acids Symposium Series.* 2005;49:311–2.
- [18] Taylor EC, Otiv SR, Durucasu I. Protection and deprotection of fused 2-amino-4(3H)-pyrimidinones: conversion of pterins and 5-deazapterins to 2,4-diamino derivatives. *Heterocycles.* 1993;36:1883.
- [19] Waring P. The synthesis of 6-aminomethyl-5,6,7,8-tetrahydropterin. *Aust J Chem.* 1988;41:667.
- [20] Gibson CL, Lang A, Ohta K, Suckling C. Specific inhibitors in vitamin biosynthesis. Part 11. Synthesis of pterins with

- extended side chains at C-6. *J J Chem Soc Perk T* 1. 1999;(2):163–70.
- [21] Taylor EC, Perlman KL, Sword IP, Sequin-Frey M, Jacobi PA. Pteridines. XXVIII. New, general, and unequivocal pterin synthesis. *J Am Chem Soc.* 1973;95:6407–12.
- [22] Pruet JM, Saito R, Manzano LA, Jasheway KR, Wiget PA, Kamat I, et al. Optimized 5-membered heterocycle-linked pterins for the inhibition of ricin toxin A. *ACS Med Chem Lett.* 2012;3:588–91.
- [23] Saito R, Pruet JM, Manzano LA, Jasheway K, Monzingo AF, Wiget PA, et al. Peptide-conjugated pterins as inhibitors of ricin toxin A. *J Med Chem.* 2013;56:320–9.
- [24] Wiget PA, Manzano LA, Pruet JM, Gao G, Saito R, Monzingo AF, et al. Sulfur incorporation generally improves ricin inhibition in pterin-appended glycine-phenylalanine dipeptide mimics. *Bioorg Med Chem Lett.* 2013;23:6799–804.
- [25] Bockman A, Pruet JM. Exploring the scope of DBU-promoted amidations of 7-methoxycarbonylpterin. *Beilstein J Org Chem.* 2020;16:509–14.
- [26] Pfeleiderer W, Zondler H, Mengel R. Pteridine, XXXIX. Synthese und Struktur von Pterin-carbonsäure-(6) und -(7). *Justus Liebigs Ann Chem.* 1970;741:64–78.
- [27] Caronna T, Gardini GP, Minisci F. Nucleophilic character of acyl radicals: a new selective type of aromatic acylation. *Chem Commun.* 1969;(5):201.
- [28] Houminer Y, Southwick EW, Williams DL. Substituent-directing effects in the homolytic acylation of pyrazine derivatives. *J Org Chem.* 1989;54(5):640–3.
- [29] Pruet JM, Robertus JD, Anslyn EV. Acyl radical insertion for the direct formation of new 7-substituted pterin analogs. *Tetrahedron Lett.* 2010;51(18):2539–40.
- [30] Taylor EC, Abdulla RF, Tanaka K, Jacobi PA. Pteridines. XXXVI. Synthesis of xanthopterin and isoxanthopterin. Application of N-oxide chemistry to highly functionalized pyrazines and pteridines. *J Org Chem.* 1975;40:2341–7.
- [31] Seeger DR, Cosulich DB, Smith JM, Hultquist ME. Analogs of pteroylglutamic acid. III. 4-Amino derivatives. *J Am Chem Soc.* 1949;71:1753–8.
- [32] Waring P, Armarego WLF. Pterins. X. A new preparation of 6-hydroxymethylpterin from 6-methylpterin. *Aust J Chem.* 1985;38:629.
- [33] Coppa F, Fontana F, Lazzarini E, Minisci F, Pianese G, Zhao L. A novel convenient and selective alkoxycarbonylation of heteroaromatic bases by oxalic acid monoesters. *Tetrahedron Lett.* 1992;33:3057–60.
- [34] Shang R, Fu Y, Li JB, Zhang SL, Guo QX, Liu L. Synthesis of aromatic esters via Pd-catalyzed decarboxylative coupling of potassium oxalate monoesters with aryl bromides and chlorides. *J Am Chem Soc.* 2009;131:5738–9.
- [35] Saraev VE, Zviagin IM, Melik-Oganjanyan RG, Sen'ko YV, Desenko SM, Chebanov VA. Green microwave-assisted multicomponent route to the formation of 5,8-dihydropyrido[2,3-d]pyrimidine skeleton in aqueous media: green microwave-assisted multicomponent route to the formation of 5,8-dihydropyrido[2,3-d]pyrimidine skeleton in aqueous media. *J Heterocycl Chem.* 2017;54:318–24.
- [36] Landauer PD, Rydon HN. A convenient synthesis of some 4-substituted 5-amino-pyrimidines. *J Chem Soc.* 1953;3706–29.
- [37] Storm CB, Shiman R, Kaufman S. Preparation of 6-substituted pterins via the Isay reaction. *J Org Chem.* 1971;36:3925–7.
- [38] Kenyon CP, Oldfield LC, Van Der Westhuyzen CW, Rousseau AL and Parkinson CJ PCT Int Appl. WO2007105023A1, 2007-09-20.
- [39] Mohr D, Kazimierczuk Z, Pfeleiderer W. Pteridines. Part XCVII. Synthesis and properties of 6-thioxanthopterin and 7-thioisoxanthopterin. *Helv Chim Acta.* 1992;75:2317–26.
- [40] Yamamoto H, Hutzenlaub W, Pfeleiderer W. Pteridine, LVIII. Synthese und Eigenschaften von Pterin- und 2,4-Diaminopteridin-mono- und -di-N-oxiden. *Chem Ber.* 1973;106:3175–93.