1 Introduction

Nucleic acids are one of the most basic substances of life, not only comprising genetic material but also playing a crucial role in protein biosynthesis. Nucleic acids are biopolymers composed of nucleotide monomers. Nucleotides in living organisms are composed of a five-carbon sugar, three phosphate molecules and a nitrogenous base (adenine, guanine, cytosine, thymine or uracil, Fig. 1). Compared with the other four bases, guanine has more unique properties because of its unique 2-amino and 6-carbonyl groups. Its 2-amino and 6-carbonyl groups can act as a hydrogen bond donor and receptor, respectively, resulting in its assembly into different structures, such as the cation-templated G-quartet and G-quadruplex.\(^1\) G-quartets have various applications in ionophore and nanostructure formation. And G-quadruplexes are present in telomerases, suppressing the growth of tumors as telomerase inhibitors. They can also take part in gel formation, and recognize proteins and small molecules. In addition, G-quadruplexes are found in promoter regions of genes and in some untranslated regions of messenger RNA (mRNA).

IsoG is a natural isomer of G, differing from G because the C2 carbonyl and C6 amino groups are transposed (purine numbering is widely used in this paper) (Fig. 1). This minor change leads to significant differences between isoG and G. isoG, also known as crotonoside, 2-hydroxyadenosine (2-OH-Ado), 2-oxoadenosine (2-oxo-Ado), 1,2-dihydro-2-oxoadenosine (1,2-dihydro-2-oxo-Ado), 2-oxo-6-amino-isoguanine-β-ribofuranoside and 6-amino-9-β-D-ribofuranosyl-9H-purine-2-oxo-isoguanine riboside. Significant research into isoG has been carried out since Fischer\(^4\) first synthesized isoguanine in 1897. isoG/isoguanine were firstly separated from natural substances such as croton beans (Crotonis oleum),\(^4\) butterfly wings\(^5\) and marine mollusks.\(^6\) Later, isoG and its derivatives were synthesized from three categories of methods, one is from the protected 2,6-diamino-9-β-D-ribofuranosylpyrimidine 1,\(^7\) adenosine 1-oxygen 2,\(^8\) the protected 6-chloroxanthosine 3 (Fig. 3)\(^9\) by the introduction of functional groups to the base. The second is from G by exchanging C2 amino and C6 carbonyl groups,\(^10\) and the third is from 5-amino-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide (AICA riboside, 4, Fig. 3) to construct the nitrogen heterocycle.\(^11\) In addition, researchers have modified the sugar or base groups of isoG/isoguanine to explore differences among them and the potential unique properties. In the meantime, the structures, self-assembly properties of isoG with/metal cations (M\(^+\)) have been studied in monomer and multi-molecular layers. isoG can self-assemble into different cation-templated supramolecular structures, such as (isoG)\(_n\)-M\(^+\) (ref. 12) and (isoG)\(_{10}\)-M\(^+\),\(^13\) in a different manner from G. Finally, its potential applications in fields of ionophore, gel-formation, and anticancer treatments etc. are also of great significance and have been systematically reviewed. We hope that this review can not only give a glimpse of the comprehensive...
development surrounding isoG, but also inspire further research into this fascinating topic.

2 Discovery process of isoG

In 1897, Fischer synthesized many purines and identified a strong similarity between guanine and one particular product, 2-oxo-6-aminopurine, which had not been discovered in nature (Fig. 2). Believing that 2-oxo-6-aminopurine was oxidized adenine, he suggested that 2-oxo-6-aminopurine would most likely be discovered in animals. And 2-oxo-6-aminopurine may have already been mistaken for guanine. In 1927, Buell et al. found that oxyadenine, a purine isolated from pig blood, is consistent with 2-oxo-6-aminopurine. However, the presence of 2-oxo-6-aminopurine in pig blood could not be confirmed. In 1932, during a study of croton seeds, Cherbuliez and Bernhard accidently discovered a new glucoside, which had not been discovered in nature. They identified it as 2-hydroxy-6-aminopurine-D-ribose. However, the yields obtained from the seeds were variable and very low, and glycosyl crystals could not be obtained by hydrolysis. Subsequently, in 1935, Spies et al. successfully enriched the new glucoside and obtained the crystals, and identified the structure as the D-ribose form. In 1939, Spies confirmed the observations of Fischer and Cherbuliez et al. that isoguanine is converted to xanthine by hydrochloric acid but is resistant to nitric acid, whereas guanine is converted to xanthine in nitric acid. This reactivity was evidence of the 2-oxo-6-aminopurine structure that Bendich had observed, i.e., that 2,6-diaminopurine can only be converted to isoguanine rather than xanthine in the reaction with excess nitrous acid. In 1939, Falconer et al. confirmed the ribose was linked to the base at N9 position. Afterwards, isoguanine was separated from the butterfly wings of Prioneris thestylis by Purrmann in 1940 and by Pettit et al. in 1976. In 1951,
experiments by Davoll et al. into optical properties of these compounds revealed that isoG was consistent with 9-β-D-ribofuranosylisoGuanine. Later, in a study of Diaulula sandiegensis in 1981, Fuhrman et al. confirmed the presence of isoG in extracts of the digestive glands of the sea slug Diaulula sandiegensis. However, the yield was very low, only one milligram of crystals can be obtained from per gram of dry tissue.

Interestingly, although isoG is a nucleoside that has been isolated from a variety of species, it does not exist as a natural nucleic acid. In 1950, Brown et al. demonstrated that isoguanine is not a precursor of nucleoside biosynthesis in mice. In 1952, they reported that isoguanine could not be used as a pure source of purines in Lactobacillus casei, nor could it be integrated into nucleic acid. Notably, C14 erotonoside has been used to investigate its role in the metabolism of Escherichia coli (E. coli). The results indicate that isoguanine and its derivatives play an important role in nucleic acid metabolism.

In 1991, using gas chromatography/mass spectrometry (GC-MS) in isolated human chromatin isoguanine, Nackerdien et al. firstly detected 2-OH-Ade within Ni(II)/Co(II)-H2O2-treated DNA. And in 1992, Dizdaroglu et al. reported in the presence of Fe(n), Cu(n), Ni(n) and Co(n), isoguanine (2-hydroxyadenine) was a product of H2O2-treated mammalian chromatin both in vivo and in vitro. Oxidation reaction commonly occurs in cells because of the presence of reactive oxygen species (ROS), which plays an important role in DNA damage. In this respect, it is logical that isoguanine is more abundant in human cancerous tissue than in normal tissue, which is reported by Olinski et al. in 1992 and 1994. In 1993, Dizdaroglu et al. identified isoguanine in mice at levels of only a few oxidation sites per 10^5 DNA bases. They also identified isoguanine as an oxidation product of hepatic chromat in exposed to γ-ray in pregnant rats and fetuses of theirs. In 1996, using GC-MS, Jaruga et al. reported that isoguanine is produced as a DNA oxidation product of adenine in E. coli, as well as in cultured human lymphoblast cells. These have drawn attention toward isoguanine as a marker for oxidative damage formation, repair, mutagenicity.20-34

In 2002, Cadet et al. demonstrated that after γ-ray radiolysis and Fenton reaction of free nucleosides, the yield of 2′-deoxyisoguanosine (isoGd) was low, compared to the yield of 8-oxo-2′-deoxy-adenosine, expect in the presence of ferrous ion (Fe2+)/ethylenediaminetetraacetic acid (EDTA) with oxygen or cuprous ion (Cu2+). However, Fe2+/EDTA with oxygen or Cu2+ are typically at very different conditions from those found in cells. They concluded that isoGd cannot be detected under a limit of per 10^5 bases in human DNA and E. coli. They also reported that the process of conventional GC-MS, which involves acid hydrolysis and derivation at high temperature, may have caused an overestimation of isoguanine/isoG. Notably, in 2019, using the ultra-performance liquid chromatography (UPLC) and electrospray ionization isotope dilution tandem mass spectrometry, Weimann et al. firstly identified and quantified the ribonucleoside isoG in mouse liver RNA, human urine, and cerebrospinal fluid (CSF). However, the amount of detected isoG is more than or equal to that of 8-oxo-G, which is most easily oxidized, and isoGd was not detected. These data are opposed to the common hypothesis that the free nucleoside isoG may originate from oxidation reaction. As a result, the reason why isoG exists in mammals still remains a matter of study.

### 3 Syntheses of isoG

As a special nucleoside, isoG is expected to present applications in various areas. However, its yield from natural sources is too low. Thus, many synthetic methods have been reported (Table 1), and these synthetic methods can be divided into three categories: (1) the introduction of the functional groups to existing heterocyclic 9-β-D-ribofuranosylpurine nucleosides

Table 1: Syntheses of isoguanine/isoG are divided into three categories: (1) the introduction of functional groups to the existing heterocycle, (2) G as a initial reactant to isoG by exchanging C2 amino and C6 carbonyl groups, (3) construction of a nitrogen heterocycle with AICA

| Year | Authors | Initial substrates | Productivity | References |
|------|---------|--------------------|--------------|------------|
| 1897 | Fischer | 6-Oxy-2,8-dichlorpurine | —a | 3 |
| 1951 | Davoll | 2,6-Diamino-9-β-D-glucopyranosylpurine/2,6-diamino-9-β-D-ribofuranosyl purine | 55% 9-β-D-GlucopyranosylisoGuanine/57% 9-β-D ribofuranosylisoGuanine | 7 |
| 1964 | Brown et al. | Adenosine 1-oxide | Variable | 8 |
| 1965 | Ravindranathan et al. | 2,6-Dianinopurine riboside | —a | 40 |
| 1968 | Montgomery et al. | The protected 2,6-diaminopurine nucleoside | By-product | 42 |
| 1995 | Napoli et al. | The protected 6-chloroxanthosine | 20% isoG | 43 |
| 1995 | Napoli et al. | 2′,3′,5′-tri-O-acetylthiamine | 76% isoG | 43 |
| 1997 | Napoli et al. | The protected 6-chloroxanthosine | 80% isoG | 48 |
| 2012 | Cheng et al. | Adenine | —a | 44 |
| 1985 | Nair et al. | G | 34% isoG | 39 |
| 1991 | Divakar et al. | G | 64% isoG | 10 |
| 1968 | Yamazaki et al. | 4-Amino-5-imidazole carboxinitrile (AICN) | —a | 45 |
| 1976 | Yamazaki et al. | AICA (riboside) | By-product | 46 |
| 1987 | Reese et al. | The protected AICA riboside | —a | 9 |
| 1987 | Chern et al. | AICA (riboside) | 77% isoG/(—a) isoguanine | 11 |

a The yield is not mentioned.
Fig. 3  Common substrates for the synthesis of isoG.

(Fig. 3), such as 2,6-diamino-9-β-D-ribofuranosylpurine \(1\), adenosine 1-oxide \(2\), the protected 6-chloroxanthosine \(3\), (2) G as an initial reactant to build isoG by exchanging the C2 amino and C6 carbonyl groups, (3) the construction of nitrogen heterocycle with AICA riboside (4, Fig. 3) as a precursor for base synthesis to introduce oxo group at C2 position. Notably, the third method is challenging because it is difficult to obtain AICA. These syntheses will be discussed in detail in the following parts.

3.1 Introducing functional groups on the existing heterocycle

Isoguanine was initially synthesized by Fischer\(^1\) in 1897. It can resist the deamination by nitrous acid, and the reaction of 2,6-diaminopurine with excess nitrous acid only yields isoguanine not xanthine. In 1951, Davoll\(^7\) et al. reported that 9-β-D-glucopyranosyl-isoguanine can be obtained in 55% yield by the reaction of 2,6-diamino-9-β-D-glucopyranosylpurine with nitrous acid, while 9-β-D-ribofuranosyl-isoguanine can be obtained in 57% yield from the reaction of 2,6-diamino-9-β-D-ribofuranosylpurine (1, Fig. 3) and nitrous acid (Fig. 4). However, the overall yields are low and harmful heavy metal ions (Hg and Pb) are used. So in 1965, Ravindranathan\(^4\) et al. synthesized isoG using the way of Davoll but using much charcoal instead of a lead salt for purification. On account of the relevance of ultraviolet (UV) light and the mutations in nucleic acids, in 1964, Brown\(^8\) et al. reported a photochemical preparation of isoG from adenosine 1-oxide (2, Fig. 4). Adenine 1-oxide is more sensitive to UV light than adenosine. However, this method gives variable proportions of two major products. One product is adenosine (6, Fig. 4) via the direct loss of oxygen. The other product is isoG via the rearrangement of oxygen atom to the neighboring carbon atom. Subsequently in 1975, Mantsch\(^4\) et al. concluded that if nucleotides of the initial substance 2 is irradiated with UV light under alkaline condition (0.01 M NH₄OH), isoG nucleotides can be the corresponding primary products. This is also the case for adenine 1-oxide (10, Fig. 5).

In 1968, in a synthesis of 2-fluoroadenosine by Montgomery\(^4\) et al., isoG was reported to be a by-product, relying on a protected 2,6-diaminopurine nucleoside (5, Fig. 4). Generally, the introduction of N to the assigned activated nucleoside bases of guanine, cytosine and adenine is easy but this is not the case for isoG. A significant intermediate 3 can be obtained from 2′,3′,5′-tri-O-acetylxanthosine 7 (Fig. 4). Nevertheless, in 1995, Napoli\(^4\) et al. reported a direct route from the protected 6-chloroxanthosine 3 to isoG, but this gives a very low yield of 20%. So, they synthesized isoG through the reagent to nucleophilic attack on the pyridinium α-carbon, and obtained the intermediate (8, Fig. 4). Then isoG was isolated by HPLC in 76% overall yield by ring opening (Zincke reaction) of the compound 8. Then in 1997, they proposed another way by introducing a strong electron-withdrawing group in the heterocyclic to enhance the reactivity of C6 towards aqueous ammonia, in
a yield of 80%, with an important intermediate (9, Fig. 4). In 2012, Cheng et al. published a new two-step method based on adenine. A mixture of isoguanine tautomers was obtained (Fig. 5). Reactions had been proved where OH radicals attack adenine on the C2, C4, C5, and C8 positions, and the reactions are spontaneous and exothermic. In the first step, attack by OH radicals at the C2 position of adenine forms a favorable low-energy complex (Fig. 5). In the second step, the enol cis–trans forms are converted into the corresponding N1H/N3H keto forms.

3.2 G to isoG by exchanging C2 amino and C6 carbonyl groups

As mentioned above, the route from 2,6-diaminopurines to isoG requires harmful heavy metal ions (Hg and Pb) and the overall yields are low. isoG was reported as a by-product in a synthesis of nucleosides from 2,6-diaminopurines, as well as from adenosine-1-oxide, both in variable yields. In 1985, Nair et al. found a new reproducible and efficient method for the synthesis of isoG starting from G (Fig. 6). This method contains five steps. Firstly, the sugar is protected by selective acetylation. Secondly, reaction with phosphorus oxychloride and N,N-dimethylaniline yields the 6-chloropurine nucleoside (11, Fig. 6). Thirdly, n-pentyl nitrite reacts with diiodomethane to obtain protected 2-ido-6-chloropurine nucleoside (12, Fig. 6). Then, 2-idoadenine (13, Fig. 6) is obtained by reacting with ethanolic ammonia. And finally, in the last key step in this process, photo-induced hydration occurs. The product isoG was isolated by HPLC. However, this method gives a low yield about 34%. In addition, in the fifth step, the requirement of UV irradiation limits the spread of this synthetic route. Therefore, in 1991, Divakar et al. developed an improved five-step route (Fig. 6) starting from G, which achieved isoG with a 64% overall yield involving the same 6-chloropurine nucleoside (11, Fig. 6), with an important intermediate 14. This means that, for the latter reaction, inavailability of direct synthesis to 6-chloroxanthosine is not required. In contrast, a better yield is obtained. Moreover, conditional complex photochemical steps are avoided.

3.3 isoG from AICA by constructing a nitrogen heterocycle

In 1968, Yamazaki et al. found that isoG/isoguanine can be obtained from 4-amino-5-imidazole carbonitrile (AICN, 16, Fig. 7) and its protected riboside (21, Fig. 7), but the yield is too low. For a long time, there have been great efforts to synthesize...
guanine from AICA. In 1976, isoguanine was discovered as an unexpected product in a synthetic process of guanine by Yamazaki et al. from AICA. Isoguanine was obtained from an intermediate substance 4-cyanamido-imidazole-carboxamide (20, Fig. 7) in weakly alkaline or neutral conditions. Whereas in alkaline conditions, the compound 20 was converted into guanine. They synthesized isoG depending on the protected AICA riboside (25, Fig. 8), in low yield with three plausible and non-isolated intermediates (27, 28, 30, Fig. 8). Subsequently in 1987, Reese et al. reported that the compound 30 can also be obtained from compound 25 via the intermediate putative methoxyacetyl thioureido derivative 29 (Fig. 8). Moreover, in 1987, enlightened by a synthesis of 1-methyl-isoG, Chern et al. proposed that an N,N,N,N-tetramethylguanidine (DCC)-mediated cyclodesulfurative method would allow milder reaction conditions and produce a higher yield of isoG. They reported an improved, more efficient route in milder reaction conditions using AICA 32 and its riboside 4 to synthesize isoguanine and isoG, respectively (Fig. 9). When AICA/AICA riboside 32/4 reacts with benzoyl isothiocyanate in dimethylformamide (DMF) at room temperature, it can be converted to benzoylthiourea intermediates 33/34, respectively. Next, DCC was added to this system and isoguanine/isoG can be obtained via stirring, washing and separation. And isoG can be obtained in a high overall yield of 77%. In 1991, they also synthesized several 1-substituted isoG through similar DCC-mediated ring-closure reactions.

In summary, as shown in Table 1, over time, the syntheses of isoG/isoguanine have developed toward milder reaction conditions and higher yield. To date, the most outstanding synthetic route to isoG is that involving 6-chloroxanthosine by Napoli et al., with a yield of 80%, under mild conditions.

4 The modified isoG

In the above sections, we have discussed the discovery of natural isoG, as well as a variety of synthetic methods. isoG contains a nitrogenous base (a purine ring) and a carbohydrate residue. Minor modification to the base or sugar may lead to great differences in the higher structures and properties compared to the parent compound. Thus, a number of base-
modified (Fig. 10) or sugar-modified (Fig. 11) analogs of isoG have been discovered in nature or synthesized. Summaries are shown in Table 2. And some of their properties are discussed in the following part.

4.1 Base-modified isoG

4.1.1 1-Methyl-isoG (doridosine). Dorid nudibranchs are shellless sea slugs. Interestingly, they have long lives despite lacking the typical protection given by a shell. Thus, there has been significant conjecture about their defense mechanism. In 1979, a tissue analysis by Fuhrman \textit{et al.} revealed that only the digestive gland extract is toxic, and this acts as the unique defense mechanism of Dorid nudibranch. In 1980, Quinn \textit{et al.} isolated a new pharmacologically active substance from the Australian sponge \textit{Tedania digitate}, in a yield of 0.71% from the crude extract. They also developed a synthesis of doridosine from AICA riboside (4, Fig. 3) by acetylation in four steps, giving a yield of 39%. Meanwhile, they identified doridosine as identical to 1-methyl-isoG (37, Fig. 10) by spectrum, chemical degradation, synthetic analysis. In 1981, Fuhrman \textit{et al.} concluded that the substances isolated from nudibranch and sponge are indeed the same. Moreover, 1-methyl-isoG 37 has been shown to have several pharmacological effects related to hypotension, hypothermia and anti-inflammatory, and these will be discussed in Section 6.3.

4.1.2 2-Methoxyadenine (spongosine). Nucleosides from marine organism have been found to have various biological activities, and this has aroused great interests among scholars. In a study of Carribean sponge \textit{Cryptotheca crypta} in Florida, a mixture of two unnatural nucleosides spongithymidine and spongouridine were obtained. And these were identified as 3-β-arabofuranosides of thymidine and uridine, respectively. The development of materials has promoted elucidation of the composition and structures of these purines and the carbohydrate. Then in 1951, Bergmann \textit{et al.} firstly isolated spongosine (38a, Fig. 10) from \textit{Cryptotheca crypta}. It was indentified as pentosylmethyl-aminooxypurine. In 1956, they determined the structure of ν-riboside of 2-methoxy-6aminopurine (2-methoxyadenine) using UV absorption spectra and the synthesis of 2-methyladenine. They concluded that it is not only the first methoxy purine found in nature, but also the first occurrence of methoxy substance isolated from animals. In addition, in 1994, Searle \textit{et al.} firstly identified 2′-deoxy-spongosine [9β-2′-deoxy-ν-ribofuranosyl]-2-methoxyadenine, 38b, Fig. 10] as a natural product from Western Australia.

4.1.3 More analogs. In terms of the great effects of 1-methylisoG 37, many analogs of 1-methyl-isoG have been prepared from the protected imidazole nucleoside to explore the structure-activity correlation. As shown in Fig. 10, some N1-substituted isoG 39 have been developed to explore the importance of N1-methyl-substituted compounds in marine products. Some of these have been synthesized in good yield, by Bartlett \textit{et al.} in 1981 and Chern \textit{et al.} in 1991. In addition, in 1981, using 1-methyl-isoG 37 as a starting material, C8-substituted-1-methyl-isoG 40 was prepared by Bartlett \textit{et al.}. Their effects on cardiovascular response and muscle relaxation, etc. will be discussed later in Section 6.3. Moreover, in 2017, Thomas \textit{et al.} identified 8-oxo-1-methyl-isoG 41a and 3′-O-acetyl-8-oxo-1-methyl-isoG 41b as natural derivatives of isoG in a sponge from the Northeastern Atlantic, \textit{Clathria (Microciona) strepsitoxa}. And these compounds showed no significant cytotoxic or antimicrobial activity. In addition, in 1994, Lee \textit{et al.} synthesized a series of 6-substituted isoG derivatives 42 and isoG by one-step nucleophilic substitution from the compound 15 (Fig. 6). These were synthesized to explore the antitumor activities, and the results will be discussed later in Section 6.3.

8-Aza-7-deazapurines and their 7-halogenated derivatives have been reported to possess excellent biological and physical properties, such as antiparasitic and antiviral effects, because of their structural similarity to purines in nucleic acids. And earlier studies showed that 7-substituents of 8-aza-7-deazapurine nucleosides and 7-deazapurine nucleosides incorporated oligodeoxynucleosides could increase antiparallel strand (aps) duplexes stability. Thus, in 2003 and 2007, Seela \textit{et al.} synthesized 7-halogenated 8-aza-7-deazaisoguanosine derivatives 43 and 7-halogenated 7-deaza-isoguanosine derivatives 44 from 8-aza-7-deazapurine-4,6-diamine riboside derivatives. Their tautomism will be discussed later in Section 5.1. Moreover, in 2004, 8-aza-3-deaza-isoG 45 was synthesized by Jeselnik \textit{et al.} to study the influence of the lack of N3 on the hydrogen-bonding modes. 8-Aza-purine nucleosides, including 8-azaadenine, 8-azaguanine, and their corresponding nucleosides, have been reported to be fluorescent. And they show various applications as fluorescent probes and in biochemical process. Consequently, in 2010, Seela \textit{et al.} synthesized 8-aza-2′-deoxoisoguanosine (z′isoGd, 46) from the protected 8-aza-2′-deoxyguanosine (z′Gd) in a yield of 27%. z′isoGd shows great fluorescence that becomes stronger in alkaline conditions than at neutral pH. Moreover, 8-azaisoguanine 47 and its N-methyl derivatives N3-, or N8-methyl-8-azaisoguanine and N3,N8-dimethyl-8-azaisoguanine 48-50 were synthesized in the method of Fox \textit{et al.}. In 2012, Wierzchowski \textit{et al.} reported these compounds 48-50 also showed
fluorescence, which is highly sensitive to the microenvironment.

4.2 Sugar-modified isoG. The structural modifications of isoG results in significant changes to its structure and functions. In addition to the previously discussed modification to the heterocycle, the glycosylation of isoG has also been studied. For example, as shown in Fig. 11, isoGd51a was first synthesized by Kazimierczuk et al. in 1973. Then in 1991, Seela et al. improved the synthesis of isoGd from 2-halogenated-isoGd, in a yield of 52–53% by photochemical methods. isoGd has been reported to be an oxidative damage product of DNA. Oxidation shows great impacts on cells, and ROS may affect multiple cellular locations, such as the connection between DNA and proteins, as well as bases mutations. In 1995, Kamiya confirmed 2-hydroxy-2'-deoxyadenosine triphosphate (2-OH-dATP) to be an oxidative product of dATP. But it is a minor product compared to 8-OH-G, which is generally believed as a significant marker of DNA oxidation, after γ-ray irradiation and exposure to the Fenton reaction.

Fluorine-substituted nucleosides have been reported to be efficient antiviral compounds. The fluorine–carbon bond is particularly stable, and its introduction to the sugar moiety can increase the lipophilicity of a drug.77 Fluorine is generally incorporated into the 2'-position of the sugar, and it has the near radius as hydrogen and can exist in duplexes. Apart from these, fluorine also has a significant effect on the sugar
conformation because of its strong electronegativity. Based on the characteristic of fluorine mentioned above, through deamination of 2,6-diamino-2'-deoxy-2'-fluoropurine nucleoside, Seela et al. first synthesized 2'-fluorine-2'-deoxyisoguanosine (FisoG, 51b, Fig. 11) in a yield of 79%. Studies concerning the stability of its duplexes will be discussed in Section 5.3.4. Some 2',3'-dideoxynucleoside derivatives have been reported to show effects such as inhibiting reverse transcription.79,80

![Fig. 11 Sugar-modified derivatives of isoG.](image)

Table 2 Derivatives of isoG referred above found in nature or by synthesis

| Analogs | Naming | From | First | Authors                          | References |
|---------|--------|------|-------|----------------------------------|------------|
|         |        |      |       |                                  |            |
| Base-modified | 1-Methyl-isoG 37 | Natural | 1979/1980 | Fuhrman et al./Quinn et al. | 49 and 50 |
|         | Spongiosine 38a | Natural | 1951   | Bergmann et al.                  | 52         |
|         | 2'-Deoxyisoguanosine 38b | Natural | 1994   | Searle et al.                    | 55         |
|         | N1-Substituted isoG 39 | Syn" | 1991/1981 | Chern et al./Bartlett et al.    | 48 and 56 |
|         | 8-Substituted-1-methyl-isoG 40 | Syn" | 1981/2017 | Bartlett et al./Firsova et al.  | 56 and 57 |
|         | 8-Oxo-1-methyl-isoG 41a | Natural | 2017   | Thomas et al.                    | 57         |
|         | 3'-O-Acetyl-8-oxo-1-methyl-isoG 41b | Natural | 2017   | Thomas et al.                    | 57         |
|         | 6-Substituted isoG 42 | Syn" | 1994   | Sung Jun Lee et al.              | 58         |
|         | 7-Halogenated-8-aza-7-deaza-isoguanosine derivatives 43 | Syn" | 2003/2007 | Seela et al.                    | 67 and 68 |
|         | 7-Halogenated-7-deaza-isoguanosine derivatives 44 | Syn" | 2007   | Seela et al.                    | 68         |
|         | 8-Aza-3-deaza-isoG 45 | Syn" | 2004   | Jeselnik et al.                  | 69         |
|         | 2'-isoG 46 | Syn" | 2010   | Seela et al.                    | 73         |
|         | 8-Aza-isoguanine 47 | Syn" | 1970   | Fox et al.                      | 74         |
|         | N3-Methyl-8-aza-isoguanine 48 | Syn" | 1970   | Fox et al.                      | 74         |
|         | N8-Methyl-8-aza-isoguanine 49 | Syn" | 1970   | Fox et al.                      | 74         |
|         | N3,N8-Dimethyl-8-aza-isoguanine 50 | Syn" | 1970   | Fox et al.                      | 74         |
| Sugar-modified | isoG 51a | Syn" | 1973   | Kazimierczuk et al.             | 76         |
|         | 5'-isoG 51b | Syn" | 2015   | Seela et al.                    | 78         |
|         | 2'-O'-Deoxy-isoG 52 | Syn" | 1999   | Kim et al.                      | 81         |
|         | 5'-[tert-Butyldimethylsilyl]-2',3'-O'-isopropylidene-substituted isoG 53 | Syn" | 1995   | Davis et al.                    | 82         |
|         | 5'-Substituted-1-methyl-isoG 54 | Syn" | 1981   | Bartlett et al.                 | 56         |
|         | 3,5-Anhydro-1-methyl-isoG hydriodide 55 | Syn" | 1981   | Bartlett et al.                 | 56         |
|         | 1-Methyl-isoG cyclic 3',5'-phosphate 56 | Syn" | 1981   | Bartlett et al.                 | 56         |

"Synthesized.
Inspired by these findings, in 1999, Kim et al. synthesized 2',3'-dideoxy-isoguanine (isoG) (52, Fig. 11) from G in a yield of 10%. And studies on its biological activities are ongoing. Besides, in 1995, the protected isoG derivatives 5'-[tert-butyldimethylsilyl]-2',3'-O-isopropylidene-substituted isoG (53, Fig. 11) has been prepared by Davis and al. to study the self-assembly of isoG. So hydrogen bonds of isoG can be researched at different temperature and in different solvents. 1-Methyl-isoG (37, Fig. 10) has been identified as a natural product. And in 1981, using 1-methyl-isoG as a starting material, Bartlett et al. prepared several N9-substituted doridosine compounds (54–56, Fig. 11). The effects of these compounds are shown later in Section 6.3. All the derivatives mentioned above can be found in Table 2 for their original sources.

5 Structure properties of isoG

The discovery of isoG and isoguanine in nature and their structural determination have been discussed above. In addition, we have discussed the low content and low extraction efficiency of these compounds in natural products, which led to the development of a series of synthetic methods. Next, some of the special structural properties of isoG, including its tautomerism at the single molecule level and the resulting unnatural pairing in nucleic acids, its self-assembled structures such as tetramers, the more highly ordered "sandwich-like" decamer structure, the isoG4 quadruplexes formed in helix, the parallel-stranded (ps) and antiparallel-stranded (aps) chains containing isoG will be discussed as follows.

5.1 Tautomerism enol–keto of isoG

The tautomeric phenomenon of nitrogen heterocycle has been widely investigated, for example, the N1H, N3H and O6H forms of guanine. In a study of poly(isoG) by Golasek et al. in 1976, the study of the tautomeric forms of isoG seems particularly important to identify correct base pairings containing isoG. In 1976, Sepiol et al. reported that the tautomerism of isoG is affected by concentration, temperature, and solvent polarity. As the polarity of the solution changes, the enol ratio can vary from 5% in aqueous medium to 80% in polar solvents. They proposed that isoG is often present in solution as a mixture of enol and keto forms. isoG shows a UV absorption 310 nm for the enol and keto forms. isoG shows a UV absorption 310 nm for the enol and keto forms. isoG shows a UV absorption 310 nm for the enol and keto forms.

5.2 Mispairing

Many researchers have studied the tolerance of base mutation that do not affect the original complementary pairing of oligonucleotides, for the purpose of expanding the genetic alphabet, designing tools for monitoring biological mechanism, and creating new gene systems. A common method is to study the pairing by inserting irregular bases. As mentioned above, isoG is an unnatural nucleoside, so the study of its base pairing is of great significance for subsequent applications. In 1962, Rich et al. hypothesized that isocytosine (isoC) and isoG, the isomers of C and G, respectively, may make up a third Watson–Crick base pair. The complementary isoG–isoC pair would form a similar geometry to that of G–C. But there were questions as to why the isoG–isoC is not a constituent of nucleic acids. Subsequently, in 1990, Benner et al. confirmed the existence of the isoG–isoC base pair, as well as a second pair, isoG–T, in vitro polymerase experiments. Afterwards, evidence for isoG–isoC pair without enzymes was obtained by Horn et al. and Switzer et al. in 1995. The base pairing of isoG in the enol form is stronger than that in the ketone form, which is the first experimental evidence that tautomerism can lead to base pairing differences. The keto–enol tautomerism of isoG and the possible cis and trans conformers result in various types of mispairing and different hydrogen-bonding modes. It has been reported that in different tautomeric forms, isoG can pair with several natural bases, deoxycytidine, deoxythymidine, deoxyuridine, deoxyguanosine, and deoxyadenosine (C9, T9, U10, G9, A9, respectively), as well as with some special unnatural bases such as isoC10 and 5-aza-7-deaza-G10 in different templates (Fig. 13). On the basis of the tautomerism of the bases described above, the possible hydrogen-bonding of isoguanine–isocytosine, isoguanine–uracil, isoguanine–cytosine were put
And in 2003, the impact of the temperature and the adjacent base pairs on base pairings of isoguanine was investigated by Maciejewska et al. They concluded both affect base-pairing by changing the polarity of the solution around the isoguanine to cause tautomeration. The temperature was found to have only a slight influence on the base pairing. The 3'-adjacent base affects the tautomeration, while the 5'-neighbor

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**Fig. 12** Tautomerism of isoG.

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**Fig. 13** Base pairs of isoG.
can not, because the 5'-terminal is not at the polymerase active site for the stacking with template.

In 1986, Jaworski et al. carried out experiments to explore the interaction energies of isoguanine–isocytosine and isoguanine–uracil, and compared them with energies of natural base pairs guanine–cytosine, adenine–thymine/adenine–uracil. It was found that isoguanine–uracil interaction energy is smaller than that of guanine–cytosine and isoguanine–isocytosine but stronger than that of adenine–thymine/adenine–uracil pairs. Additionally, isoguanine–isocytosine pairing is slightly stronger than guanine–cytosine pairing. And isoGd5-methyl-isoCd pair was reported to be isoenergetic with the G–C pair, and each base can effectively identify mismatches in synthetic oligonucleotides, by Horn et al. in 1995 and Switzer et al. in 1997. The isoguanine–cytosine and uracil–adenine base pairs have the same energy. In 1997, using ab initio data from calculations combined with thermodynamic data, Switzer et al. concluded the reason why isoguanine–isocytosine shows unnatural stability may be that isoguanine adopts a carbonyl-imino form.

Isoguanine is a modified base, so its enzymatic recognition and removal have aroused wide interest among scholars. Various DNA and RNA enzymes can catalyze the recognition of different bases by hydrogen bonding interactions. Thus, the enzymological properties of these varied base pairs have been investigated to explore whether they are incorporated into duplexes by DNA and RNA polymerases, and, if so, under what conditions. It has been demonstrated that various enzymes catalyze the entry of isoG into the nucleotide chains in vitro (Table 3). For the first time, Switzer et al. reported that d-isoGTP can be incorporated opposite T/isoC by the Klenow fragment (KF) of E. coli DNA polymerase (pol) I and T7 RNA polymerase in 1989. Then in 1993, they reported that in different templates, avian myeloblastosis virus (AMV) reverse transcriptase can enhanced the formation of isoG–isoC, and isoG–U base pairs via T7 RNA pol. And T7 RNA pol has a preference in catalyzing the incorporation of U over isoC. However, T4 DNA pol rejected isoguanine’s incorporation under reported experimental conditions. Moreover, in 1993, Dervan et al. reported that 5-methyl-isoCd can direct the incorporation of N6-(6-aminohexyl)-isoG into the transcribed RNA by T7 RNA pol. And this amino group affects site-specifically modified RNA sequences beneficial for future studies after transcription. Until then, the possibility of isoG opposite A/G and the incorporation by mammalian pol were not investigated. Subsequently, in 1995, in vitro DNA synthesis, Kamiya et al. reported that deoxythymidine monophosphate (dTMP) and deoxyadenosine monophosphate (dAMP) can be incorporated to pair with isoG by KF of E.coli DNA pol I, recombinant rat DNA pol β and calf thymus DNA pol α. And deoxyguanosine monophosphate (dGMP) can pair with isoG by KF of E.coli DNA pol I. They also concluded that d-isoGTP can mismatch with T/C (showing a preference for T) using mammalian (calf) DNA pol α.29 Whereas d-isoGTP can only pair with T by KF of E.coli DNA pol I. In 1996, using calf thymus DNA pol α and recombinant rat DNA pol β, they confirmed the dTMP and deoxyctydine monophosphate (dCMP) pairs with isoG.103 Whereas dTMP and dGMP were incorporated to form base pairs with isoG by KF of DNA pol I. In addition, human immunodeficiency virus 1 reverse transcriptase (HIV-1RT) has a high tolerance for nonstandard bases, indicating that it promotes nonstandard base pairing containing isoGd, and thus preventing HIV-infected cells from being recognized and dying in the cell’s S phase.104,105 Then in 1998, Lutz et al. reported that compared to eukaryotic DNA polymerases, the incorporation of T or U by HIV-1RT to mismatch with d-isoGTP is more efficient, whether dATP exists or not. In 2000, Kamiya et al. reported the 2-OH-dATP can pair with T and G using the α (catalytic) subunit of E. coli DNA pol III and KF of DNA pol I. Above all, the incorporation of isoG depends on the types of polymerases, as well as the sequence contexts in various templates.

In 1975, Mantsch et al. reported that isoguanine nucleotides are not recognized by the mitochondrial endomembranial location enzyme system, but they can be well recognized by mitochondrial phosphatases in the inter-membrane space. They are also involved in phosphor group transfer reactions. These reactions are catalyzed by pyruvate kinase, phosphofructokinase and hexokinase. Additionally, in 1998, Kamiya et al. reported that isoguanine opposite thymine/cytosine cannot be moved by a glycosylase-type mechanism in rat organs. In bacteria such as in E. coli, there are some specific enzymes for the removal of unnatural base from damaged nucleic acid. In 2011, Rausch et al. reported that cytosine deaminase (CDA) from E. coli can catalyze the deamination of isoguanine to xanthine. However, in 2000, Kamiya et al. demonstrated that the removal of isoguanine by the MutY and MutM proteins of E. coli is slow because of poor affinity. Surprisingly, they reported that among the known akin nucleotide substrates, the nucleotide forms of isoG (2-OH-ATP and 2-OH-dATP, where ATP is adenosine triphosphate and dATP is deoxyadenosine triphosphate) are substrates with the highest affinity for the human MTH1 (hMTH1) protein, which is a hydrolytic enzyme.111,114 Moreover, 2-OH-dADP and 8-OH-dGDP (dADP and dGDP are deoxyadenosine diphosphate and deoxyguanosine diphosphate, respectively) can competitively inhibit the effect of hMTH1 on 2-OH-dATP and 8-OH-dGTP (dGTP is

| Enzymes | E. coli DNA pol I | E. coli DNA pol III | DNA pol β | DNA pol α | T7 RNA pol | AMV reverse transcriptase isoC | T4 pol | HIV-1RT |
|---------|-----------------|-------------------|-----------|-----------|-----------|-----------------------------|-------|--------|
| Mispairing with isoG | T/isoC/A/G | T/G | T/A/C | T/A/C | U/T/isoC | — — | T/U |
| References | 29, 31, 100, 103, 107 and 108 | 107 and 108 | 31 and 103 | 29, 31 and 103 | 100 and 101 | 101 | 101 |

*T4 pol do not catalyze any nucleosides opposite isoG under experimental conditions.*
5.3 Self-assembling of isoG

We have discussed the tautomeric forms of isoG in different solvents and the various base pairs catalyzed by different DNA and RNA enzymes above. Now, we will describe several types of advanced structures such as tetramers, decamers, oligonucleotides, and ps/aps strands that form based on the self-assembly properties of isoG. Self-assembly is a process where substances combine into a supramolecular system through intermolecular forces, such as electrostatic and hydrophobic interactions. The discoveries of poly(isoguanylic acid), the oligonucleotide d(T4-isoG,T4), and G-quartets indicate that isoG may also self-assemble into supramolecular structures. In fact, both G and isoG can self-assemble into supramolecular systems, such as supramolecular gel by hydrogen-bonding, π–π stacking, and hydrophobic interactions. And these structures show a wide range of applications in material science, medical chemistry, and nanotechnology etc. As mentioned before, isoG differs from G in that the C2 carbonyl and C6 amino groups are transposed. This minor change causes significant differences between isoG and G. In particular, the variation in hydrogen bond donors and acceptors affects the hydrogen-bonded systems, resulting in great changes in self-assembled supramolecular structures. For G, the angle between the hydrogen bond donor (N1 amide and N2 amino) on its Watson–Crick face and the receptor (N7 and O6) in the Hoogsteen face is 90°, and this is favorable for the formation of planar tetrads (Fig. 14).115,116 On the contrary, the isoG donor–receptor angle of 67° prefers cyclic pentad (Fig. 14). In 1994, Davis et al. raised that for isoG, there are two prerequisites for self-association. One is that the hydrogen-bonding receptors of isoG (O2–C2–N3) are close to 180°, whereas the N7–C5–C6–O6 bond of G is nonplanar. And the ribose of isoG, as well as the hydrogen-bond acceptors, are more likely to form hydrogen bonds in the lower position of purine.117,118 The other prerequisite is the formation of hydrogen bond between the N6-proton and adjacent isoG monomer O2’. In contrast, G is unlikely to form such a hydrogen bond because the hydrogen bond acceptor O6/N7 and the glycosyl group are in opposite positions in the heterocycle.82 This is also an important reason why isoG self-assembled oligonucleotides are more stable than the corresponding G self-assembled oligonucleotides.119 It has also been proposed that isoG has three variable N protons that are important for supporting the self-assembly.82 C6NH3 and C6NH2 (Fig. 14) rotates slowly, allowing the exocyclic amino group to form a strong hydrogen bond, and the third proton N1H is confirmed based on nuclear Overhauser effect (NOE) and UV studies in CDCl3.82

5.3.1 The tetramer model of isoG. As shown in Fig. 14, without cations, some free G lipophilic derivatives such as Gd can only self-assemble to different ribbons by taking N3 as a hydrogen-bonding receptor.120 And in 2002, Gottarelli et al. reported that some lipophilic derivatives are potential gelators. However, for isoG, self-assembly into tetramers or decamers without cations is possible. In the presence of cations, G-tetramer was identified as the basis of the self-assembly of G.121 It is widely accepted that G-tetramer takes shape via eight Hoogsteen hydrogen-bonds, and a central cavity containing four oxygens that bind to cation through ion–dipole interactions. The stability of G-quartets varies with different radii and charges of the cations. In 1978, Becker et al. reported that G-quartets are stabilized in the presence of sodium ion (Na⁺) and potassium ion (K⁺). In 1994, using 2D nuclear Overhauser spectroscopy (NOESY) and 1D NOE experiments, the isoG tetramer, a basic unit of isoG self-assembled structures, was confirmed. When two nuclei (A and B) are close to each other in space and have strong mutual relaxation, NOE occurs. This means that when A is saturated by irradiation, its energy is transferred to B, so that the energy absorbed by B increases and the resonance signal also increases. The specific NOEs between the exocyclic C6NH2
amino proton and the adjacent ribose H1' and H2' protons are confirmation of isoG self-association. Only the isoG tetramer model conforms with the observed NOEs.

Similar to the interaction of the cations with G, alkali ions have a great influence on the self-assembled structures of isoG. Different ions have been titrated into pure isoG mononucleoside in d6-acetone to identify the different structures. Tetramerization with lithium ion (Li+) is unstable because Li+ is too small for the central cavity. Li+ may be combined with the O2 or N3 hydrogen-bond donors to hinder hydrogen-bond formation. A low concentration of Na+ can stabilize the tetramer, probably via ionic coupling. But no new NMR peaks have been observed with Na+, which indicates that no higher-ordered self-assemblies, such as stacking structures, are formed in the presence of Na+. Nevertheless, high concentrations of Na+ destroy isoguanine tetramers, probably for the same reason as that for Li+. Notably, the addition of K+/barium ion (Ba2+) form octamers, presenting a new peak with a sandwich structure. And K+/Ba2+ enhanced the thermal stability compared to the original tetramer. Moreover, the Ba2+ octamer is more stable than K+ octamer.

The hydrogen-bonding modes are different in the isoG tetramer in the presence and absence of cations. Next, the hydrogen bonds in alkali-metal-free isoG tetrad and pentad structures will be introduced. In 2003, Meyer et al. proposed that the hydrogen-bond modes in tetrad and pentad are different (Fig. 14), one difference is that the N1–H1···O2 hydrogen bond (H bond) in the tetrad is longer than that in the pentad. The other is the tetrad N6–H6···O2 H bond forms a crossing hydrogen bond with the N1–H1···O2 H bond. Whereas two hydrogen bond, N1–H1···O2 and N6–H6···N3 are formed without crossing in the pentad. Besides, the second H bond N6–H6···N3 in pentad is shorter than the second bond N6–H6···O2 in tetrad. When the ions are added to the isoG solution, there are no crossing hydrogen bonds occurring. In other words, alkali ions can affect the crossing hydrogen-bond mode of the protected isoG tetramer (53)_4 to form independent N6–H6···N3 and N1–H1···O2 hydrogen bonds (Fig. 14). This is not surprising because base-cation linkages are usually stronger than base–base linkages for isoG. That is, the cation interactions are the dominant contribution to the interaction energy. As a result of metal cations present in the cavity, the base–base hydrogen bond in the tetrad is shorter than in the pentad, and the distance between cation and O2 is also shorter. It indicates the hydrogen bond distance is shortened in the presence of alkali ions, and this effect is more pronounced for Li+ and decreases with increasing ion size. From Li+ to cesium ion (Cs+), the distance between O2 and the cation increases. Further, larger ions result in smaller connection energies between the ion and C2 carbonyl group.

The protected isoG tetramer (53)_4·M+ is stabilized by the hydrogen-bond system of the outer ring and the inner ring (Fig. 14). The inner ring is composed of four hydrogen bonds formed between the imino N1H proton and the C2 carbonyl oxygen of an adjacent isoG. The outer ring is comprised of hydrogen bonds between N6H and N3H of the adjacent monomer. Four nonbonded C2 oxygen atoms point to the cavity, interacting with metal alkali ions, and selectively bind to monovalent or divalent cations to form a stable complex. The presence of multiple metal ions causes different changes to hydrogen-bond model, and metal cations are often required to stabilize hydrogen-bonded macrocycles. The self-assembled structures of isoguanine with different metal ions varies. For example, the tetramer of isoG is strongly nonplanar except with Li+, whereas the pentamer is often planar. In fact, most nonplanar structures converge into planar structures, so most of the known tetrad/pentad structures are planar, which is more stable. However, there is no selectivity over monovalent and divalent ions. That is, there is no difference in affinity for different charge numbers. Moreover, the tetrad has strong selectivity and preference for small ions. However, for large ions, there is no preference between the tetrad and pentad. The energy of [isoG]4·Li+ tetrad is higher than that of its pentad, whereas the energy of the pentad with a larger radius is higher.
than the corresponding tetrad energy. The energies of the planar tetrad structures are relatively low; i.e., they are more stable. And the metal-free and (isoG)₅·K⁺/rubidium ion (Rb⁺) pentad energies are also relatively low. Li⁺ and Na⁺ ions are too small for the pentameric cavity, whereas Cs⁺ is too large to be in the cavity so it may sit over the isoG₅ pentad plane. The protected isoG (53, Fig. 11) can also self-associate in the gas phase and studies into this fact are ongoing.

5.3.2 Sandwiched structure formed by isoG. In 2000, by X-ray crystallography, Davis et al. showed that the protected G (57, Fig. 15) can stack into an ordered hexadecamer. And solid-state decamers have been obtained with Ba²⁺ and strontium (Sr²⁺) in 2001 by his group. Anions are omitted for clearness. They also showed the role of organic anions in the cation-filled quadruplexes, which predominate in solution. In 2000, they reported isoG, an isomer of G, can also stack into a sandwiched structure (53)₁₀·Cs⁺ (Fig. 15), comprising two hydrogen-bonded isoG pentads. This structure forms in both solution and solid state. Two-side views of the crystal sandwich structure are shown (Fig. 16): a looking view showing the two pentameric tail–tail connections that directly overlap with ten chemically equivalent isoG units having Cs⁺ and ten carbonyl groups in the center; the side view shows a sandwich model with Cs⁺ as the bisector of the plane. In 1999, depending on conventional NMR, Davis et al. concluded that G and isoG self-assemble independently in the presence of Cs⁺, which reflects the fact that the cavity size is more suitable for the large radius of Cs⁺. In addition to the above, in 2002, from the NMR spectrum, Davis et al. concluded that G and isoG self-assemble independently in the presence of Cs⁺/Ba²⁺, rather than form crosslinked structures (Fig. 15). However, in Cd₂Cl₂ where there is no cation, G and isoG are crosslinked.

Moreover, in 2000, Davis et al. found that (n,n)-isoG (53, 59, Fig. 17) undergoes enantiomeric self-recognition in solution containing Cs⁺. Then in 2001, they found that the protected (n,n)-G (57, 58, Fig. 17) can self-associate with diastereoselectivity in Ba²⁺-containing solutions, yielding two homochiral G-quadruplexes (57)₁₆·2Ba²⁺·pic and (58)₁₆·2Ba²⁺·4pic. But in the presence of K⁺, (n,n)-G forms a diastereomeric mixture. As mentioned above, hydrogen bonds can take shape between neighboring isoG ribose residue to help conquer the entropic demands in relation to enantiomeric self-sorting, whereas this does not occur for G. Such hydrogen bonds can transfer conformational information between pentameric isoG to ensure homochiral conformation. And the achiral Cs⁺ promotes the enantioselectivity of isoG, thus promoting formation of the Cs⁺ sandwich meso decamer, (53)₃·Cs⁺·(59)₃·Ph₄B⁺, as determined by X-ray crystal structure analysis. However, enantioselectivity is not possible with other ions.
5.3.3 Oligonucleotide aggregates d(T₄isoG₄T₄)₄. The tetramer and decamer structures of isoG have been summarized above, next, the isoG-based aggregated structures in duplexes will be talked about. In 1984, Blackburn et al. reported that some [G₄]₄ aggregates are natural parts of telomeres. This has aroused great interest in G-quadruplexes. In 1989, Williamson et al. found that oligonucleotides including short runs of G, such as d(T₄G₄T₄), can form four-stranded nucleic acid structure named G-quadruplexes. And the fundamental structure is the G-quartet composed of four G units interacting via Hoogsteen hydrogen bonds in planar (Fig. 14). G-quartets stacked in a helical mode via π–π interactions are stabilized by cations such as K⁺ and Na⁺ (preference K⁺ > Na⁺). And with cations linked with O6 of G in the interior channel, G-quartets show a parallel/antiparallel orientation of strands. The oligonucleotides containing short runs of G can self-assemble into higher aggregates, which can be classified depending on their molecularity [i.e., unimolecular (monomer), bimolecular (dimer), and tetraplex (tetramer)] or in terms of their strand orientation (i.e., chair or basket) (Fig. 18a–f). In 1996, based on ion-exchange HPLC and circular dichroism (CD) spectroscopy, Seela et al. demonstrated the identification of an isoG-containing oligonucleotide quartet d(T₄isoG₄T₄), which is more stable than d(T₄G₄T₄). This conclusion also applies to the comparison of thermal stability of their tetraplexes, i.e., d(T₄isoG₄T₂)₄ is more stable than d(T₄G₄T₂)₄. Subsequently, in 1997, they proved that the stability of d(T₄isoG₄T₂) against exonuclease hydrolysis is much higher than that of d(T₄G₄T₂). The same is true for their tetraplexes. In general, base residues can pair to cause reverse trimerization or tetramerization, but when the increasing energy arising from stacking or hydrogen bonding is greater than the energy lost because of the main degeneration, the double helix structure is generally more stable, and still able to maintain a stable Watson–Crick base pair model.

In 1997, by native polyacrylamide gel electrophoresis (PAGE), Roberts et al. reported that similar to G, isoG also shows a tendency to form tetraplexes, showing the similar monomer, dimer, tetramer structure (Fig. 18a–f). Notably, they found that isoG can even form mixed quartets with G, which may induce the formation of mixed tetraplexes. Moreover, isoG-tetraplex may show higher stability than G-tetraplex in the presence of K⁺. And isoG-tetraplex shows a preference for K⁺ over Na⁺. Afterwards, in 1998, Seela et al. concluded that oligonucleotide tetraplex d(T₄isoG₄T₂)₄ has an ion preference in the following order: K⁺ > Rb⁺ > Na⁺ > Cs⁺ > Li⁺. In contrast, the order for d(T₄G₄T₂)₄ is K⁺ > Rb⁺ > Na⁺ > Cs⁺ > Li⁺. In 1999, Switzer et al. reported that ionic identity can determine the self-assembly of DNA strands into quadruplexes or pentaplexes. In the presence of Cs⁺, isoG self-assembles into pentaplexes. Whereas, in the presence of Na⁺ and K⁺, it forms quadruplexes. However, it was found that G self-assembles into almost the same quadruplexes in Cs⁺ and K⁺, which is consistent with the results of Sen et al. in 1993. Interestingly, in 2001, Seela et al. found that 8-aza-7-deaza-isoGd (43a, Fig. 10) can self-assemble into d(T₄(43a)₄)₄. And the compound 43a can form a pentaplex (Fig. 18g) in the presence of Cs⁺, and a tetraplex in the presence of Na⁺, K⁺ and Rb⁺. This is different from d(T₄isoG₄T₂) because the d(T₄isoG₄T₂) can self-assemble into both four-stranded and five-stranded structure in Rb⁺ solution. In addition, in 1997, they reported that 7-deaza-isoGd (44a, Fig. 10) can also form a tetraplex, identical in hydrogen-bonding structure to d(T₄isoG₄T₂)₄, but the former is less stable because of the heterocycle change. This observation is also a confirmation that N7 does not participate in the isoG hydrogen-bonding system. Moreover, in 1997, they proved that isoGd is sensitive to acid and can be easily hydrolyzed under acidic conditions. But 7-deaza and 8-aza-7-deaza purines can add to the stability of the glycosyclic bond. Moreover, 7-deaza-isoGd can form stronger tetraplexes in the presence of Na⁺/K⁺ comparing to isoGd, respectively. The 7-halogen substitution is capable of enhancing stabilities of the base pairs (even in mismatches), glycosyclic bond, and duplexes of 8-aza-7-deaza-isoG derivatives. However, 7-deaza-isoGd show the topmost glycosyclic bond stability but the lowest duplex stability.

5.3.4 Parallel-stranded (ps) and antiparallel stranded (aps) chains containing isoG. A high Apd–Tpd content and the special sequences are prerequisites for ps DNA duplexes. In general, ps DNA is more unstable than the aps counterparts, because of weaker stacking interactions and hydrogen bonds by Apd–Tpd reversed Watson–Crick (Donohue) base pairing. G and 5-methyl-isoC base pair incorporated with A–T can lead to ps duplexes with lower stability than the corresponding aps duplexes (Fig. 19). Ps DNA can be found in some oligonucleotide duplexes, triplexes and some tetrameric aggregates. In 1993, Seela et al. initially reported that the oligonucleotides containing isoG–C can form a new type of ps DNA duplexes (Fig. 19). And then, in 1999, they proposed that if a central Apd–Tpd is replaced by a isoGd–Cd, the stability of the original aps duplex will be reduced. Further, if isoGd–Cd replaces two Apd–Tpd pairs, it will form the ps chains. However, oligonucleotides containing isoGd–isoCd still keep aps duplexes (Fig. 19). Notably, in 2013,
they reported that tridentate isoGd=Cd base pairs can be used to compensate for the loss and stabilize ps DNA. In addition, in 2003, they proposed that 7-halogenated 8-aza-7-deaza-isoGd (43b, 43c, Fig. 10) can significantly increase the duplex stability of 8-aza-7-deaza-isoGd (43a) both in ps and aps DNA. In 2015, they raised that FisoGd (51b, Fig. 11) can add to the stability of aps duplexes, and this effect correlates to the number of incorporated FisoGd units. But this is not true for ps duplexes because the sugar conformation of FisoGd does not apply to ps duplexes.

6 Applications

In the above parts, the discovery of isoG, its synthesis, modifications, tautomerism, and different mismatches, as well as the self-assembly properties of isoG, have been introduced. Based on the above characteristics, the applications of isoG have been investigated, such as the formation of supramolecular gels, the role of base-pairing in duplexes, its use as an ionophore, and its use as an antitumor agent. In the following sections, these applications will be discussed in detail.

6.1 isoG-based supramolecular hydrogels

Hydrogels have been an important topic for centuries because of their diverse applications in many fields, including in nanoscience, medicine, and the cosmetics industry. Hydrogels are viscoelastic solid-like materials composed of an elastic crosslinked network, and the major component solvent. Hydrogels can be divided into two categories. One is polymer hydrogels, whose shape is maintained by strong chemical bonds crosslinked long chains, which cannot be broken easily and are not thermally reversible. The other one is low molecular weight hydrogels (LMWH). LMWH formed by the self-assembly of materials through noncovalent interactions, such as hydrogen-bonding, π–π stacking, hydrophobic forces, and van der Waals interactions. LMWH, also known as supramolecular hydrogels or physical hydrogels, will be discussed in this paper. Nucleosides are desirable candidates for building...
supramolecular hydrogels because of their ability to self-assemble via noncovalent interactions, as well as their biocompatibility in cells.

The gelation of guanylic acid was firstly identified in 1910 by Bang.\textsuperscript{134} G and some of its derivatives can self-assemble into various supramolecular gels based on its unique hydrogen bond donors and receptors.\textsuperscript{134} There have been many reports concerning G-based hydrogels and their applications in diagnostics and nanoscience. But these G-based hydrogels all show short lifespans. So finding new nucleosides that can form long-life supramolecular hydrogels is crucial. Like G, isoG can form gels as well. In a synthesis of isoG in 1951, Davoll\textsuperscript{7} noted the formation of a “gelatinous material” during the deamination of 2,6-diaminopurine, but he did not characterize this material further by biochemistry. It was preliminarily described that the aggregation state of nucleosides turns viscous at higher concentrations and when cooled. Then in 1965, Ravindranathan\textsuperscript{48} et al. synthesized isoG and gave proof for the phenomenon observed by Davoll.\textsuperscript{7} They reported the formation of asymmetry, ruled, ordered, spiral and temperature-depending structures where the nucleosides were unchanged. And these structures are similar to the gel formed by 5’-monophosphate-guanosine (5’-GMP), as identified by infrared spectroscopy. However, the preference of isoG for alkali ions is different from that of G. They also concluded that the purine ring may be hydrogen-bonded with the amino group of another di- and tri-pyrimidine nucleosides. The phenomenon observed by Davoll was further confirmed by biochemistry. It was preliminarily described that the aggregation state of nucleosides turns viscous at higher concentrations and when cooled.

As shown in Table 4 and Fig. 20, remarkably, in 2017, Seela\textsuperscript{155} et al. found that isoG-based hydrogels formed in various solutions containing Li\textsuperscript{+}, Na\textsuperscript{+}, K\textsuperscript{+}, Rb\textsuperscript{+} and Cs\textsuperscript{+}, but not in water. And all hydrogels present significant longer lifespans (two or three months) compared to those of G (several days), as well as higher thermodynamic stability and wider pH range. The rheological results suggested that the stabilities of isoG-based gels are strikingly higher than those of G. And scanning electronic microscope (SEM) images revealed that isoG-based hydrogels form helix-like cylinders, whereas G-based gels form individual flat ribbons. As reported, hydrogels formed by isoG at physiological Na\textsuperscript{+} concentration showed a good small molecule loading and releasing capacity in phosphate buffered saline (PBS) buffer. This suggested their potential applications in drug delivery and as ionophores carrying different ions. In addition, supramolecular hydrogel systems of isoG\textsubscript{d} (51a, Fig. 11) solutions containing Li\textsuperscript{+}, K\textsuperscript{+} and Rb\textsuperscript{+} have been established. However, in Na\textsuperscript{+} and Cs\textsuperscript{+} solutions, these hydrogels can not take shape. Moreover, the derivative \textsuperscript{5}isoG\textsubscript{d} (51b, Fig. 11) can also form supramolecular hydrogels in the presence of Li\textsuperscript{+}, Na\textsuperscript{+}, K\textsuperscript{+} and Rb\textsuperscript{+}. While in solutions containing Cs\textsuperscript{+}, precipitates form, indicating the ion dependence of the three nucleosides-based gels is different.

Hydrogels with fluorescence have aroused much attention due to their latent applications in drug delivery, molecular marking, and tissue engineering. As discussed before, z\textsuperscript{8}isoG\textsubscript{d}, z\textsuperscript{8}G\textsubscript{d} are virtually fluorescent in alkaline environment and at neutral pH, similar to those reported for 8-aza-G and relevant 8-azapurine nucleosides.\textsuperscript{78-73} Notably, isoG\textsubscript{d} and G\textsubscript{d} are not fluorescent. In addition, z\textsuperscript{8}isoG\textsubscript{d} and z\textsuperscript{8}G\textsubscript{d} both show self-assembly properties. So experiments have been carried out by Seela\textsuperscript{155} et al. in 2017 to prepare smart (stimulus-responsive) gels with fluorescence, which can be regulated by external changes, such as pH, heat and nucleoside mimics. Remarkably, z\textsuperscript{8}isoG\textsubscript{d} formed the first G-similar gelator with fluorescence properties even in solid state. And the hydrogel also shows a longer lifetime and higher thermal stability than isoG and G hydrogels. However, z\textsuperscript{8}G\textsubscript{d} does not form a gel under experimental conditions. z\textsuperscript{8}isoG\textsubscript{d} hydrogel has a great selectivity for K\textsuperscript{+}, and its pentad and tetrad structures are the basis of its self-assembly into gels. SEM images revealed that z\textsuperscript{8}isoG\textsubscript{d} gel forms dense fibers and bundles, whereas z\textsuperscript{8}G\textsubscript{d} forms nanotubes. It makes great significance that the fluorescence hydrogel formed by z\textsuperscript{8}isoG\textsubscript{d} can response to external changes such as pH, UV, and heat. Additionally, in terms of the ability to form gels even at a very low KCl concentrations, z\textsuperscript{8}isoG\textsubscript{d} hydrogel is expected to be applied in various fields of nanobiotechnology, chemistry, biology and medicine. Interestingly, although N7 does not participate in the isoG hydrogen-bonding system, the absence of N7 in 7-deaza-isoG\textsubscript{d} results in this system being unable to form a hydrogel. Moreover, the addition of 1.0 equivalent of 7deaza-isoG\textsubscript{d} can break the hydrogels formed by isoG and the fluorescence hydrogels formed by z\textsuperscript{8}isoG\textsubscript{d}.

Self-healing is an ability to self-repair when the external stimuli, such as pH, external force and temperature, are moved. It is one of the most attractive properties of hydrogels and empowers them with diverse applications in many fields like tissue engineering. For example, so-called injectable hydrogels undergo shear-thinning on injection, but the hydrogels recover soon after injection.\textsuperscript{157} There have been many reports on self-healing polymer hydrogels, but only a few studies concerning the self-healing supramolecular nucleoside hydrogels have been published. In 2018, Das\textsuperscript{158} et al. developed a self-healing arylboronate esters mediated G-quartet hydrogel as promising 3D-bioink. However, the applications of G-based hydrogels are limited for their short lifetime within hours and low stabilities. This has drawn attention to isoG, an isomer of G. However, the injection of isoG hydrogels is challenging.\textsuperscript{159} Recently, inspired

### Table 4 The property of supramolecular gel forming by isoG, its derivatives and G–isoG co-gel

|   | H\textsubscript{2}O | Li\textsuperscript{+} | Na\textsuperscript{+} | K\textsuperscript{+} | Rb\textsuperscript{+} | Cs\textsuperscript{+} |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| isoG | S\textsuperscript{c} | Gel | Gel | Gel | Gel | Gel |
| G | — | Cr\textsuperscript{b} | Cr\textsuperscript{b} | Cr\textsuperscript{b} | Cr\textsuperscript{b} | Cr\textsuperscript{b} |
| isoG\textsubscript{d} | Gel | Gel | S\textsuperscript{c} | Gel | Gel | S\textsuperscript{c} |
| G\textsubscript{d} | Cr\textsuperscript{b} | Cr\textsuperscript{b} | Cr\textsuperscript{b} | Gel | Vs\textsuperscript{c} | Cr\textsuperscript{b} |
| iisoG\textsubscript{d} | Gel | Gel | Gel | Gel | Gel | S\textsuperscript{c} |
| z\textsuperscript{8}isoG\textsubscript{d} | Pre\textsuperscript{a} | Pre\textsuperscript{a} | Pre\textsuperscript{a} | Gel | Pre\textsuperscript{a} | Pre\textsuperscript{a} |
| z\textsuperscript{8}G\textsubscript{d} | Pre\textsuperscript{a} | S\textsuperscript{c} | S\textsuperscript{c} | S\textsuperscript{c} | S\textsuperscript{c} | S\textsuperscript{c} |
| G : isoG (1 : 1) | — | Pre\textsuperscript{a} | Cr\textsuperscript{b} | Gel | Gel | Cr\textsuperscript{b} |
| G : isoG (1 : 3) | — | Cr\textsuperscript{b} | Cr\textsuperscript{b} | Gel | Gel | Cr\textsuperscript{b} |
| G : isoG (3 : 1) | — | Cr\textsuperscript{b} | Cr\textsuperscript{b} | Gel | Cr\textsuperscript{b} | Cr\textsuperscript{b} |

\textsuperscript{a}Precipitation. \textsuperscript{b}Crystallization. \textsuperscript{c}Solution. \textsuperscript{d}Viscous solution. \textsuperscript{e}Not experimented.
by the self-assembled tetramers formed by mixing d(T₈isoG₄T) and d(T₄isoG₄T) verified by Seela et al. in 1996, the tetraplexes formed by d(T₈isoG₄T) and d(T₄G₄T) in the presence of K⁺ proved by Roberts et al. in 1997, and the self-healing co-gel developed by mixing G and isoG with 1:1 and 1:3 molar ratios in the presence of K⁺. In terms of ion dependence, both mixtures can form co-gels in the presence of K⁺ and Rb⁺. But the 3:1 could only produce a co-gel in the presence of K⁺. Furthermore, the lifespan of the gels varied from hours to months. SEM images show that these co-gels form flower-like structures. Rheological and injection experiments concluded the excellent self-healing properties, short recovery time, and syringeability of the co-gel. Moreover, G isoG₂-quad- titers are possibly formed inside the self-healing supramolecular nucleoside co-gel.

Furthermore, it is widely known that isoG possesses antitumor property toward several cancer lines (details are shown in Section 6.5); it is well-known as a dynamic covalent bond to construct self-healing hydrogels. Based on these, in 2017, Sadler et al. developed an anticancer hydrogel (dopamine-conjugated platinum IV-G₄K⁺-borate ester, Pt-G₄K⁺B hydrogel) by using borate ester linkage. Inspired by the above-mentioned findings, recently, Zhao et al. developed another type of dual-functional co-gel called isoguanosine-borate-guanosine (isoGBG) hydrogel (Fig. 21), which possesses delivery and antitumor activities. isoGBG hydrogel displays excellent stability, self-healing property and biocompatibility, as well as highly antitumor activity and excellent inhibition of tumor recurrence in vivo. Moreover, the mechanism was preliminarily explored, and it was concluded that the mechanism of inhibiting oral cancer cells might be through the caspase-dependent signaling pathway to induce apoptosis via regulating dephosphorylation of epidermal growth factor receptor (EGFR) (Fig. 21).

6.2 Applications of isoG mispairing

In Section 5.2, different mismatches and the incorporation of isoguanine have been discussed. Although isoG can be integrated into DNA/RNA by multiple enzymes in various templates, isoG–isoC is not accepted as a natural base pair. The three reasons why isoG–isoC cannot form an R-type triple-chain structure. Although the tautomerism of isoG limits its stable transmission as a gene letter, it enables the existence of gene mismatch, suggesting isoG's various alternative applications. In 1996, for the first time, Kamiya et al. suggested that isoG may be mutagenic and lead to A to C, A to G and A to T transversions in cells. Then in 1997, isoG is proved to induce mutagenicity in E. coli, as well as in living COS-7 cells, but it does not hinder the replication. These indicate the mutation caused by isoG can occur in both eukaryotic and prokaryotic cells. Interestingly, the mutagenicity is affected by the sequence and strands that the base is located in. 8-OH-dGTP is a significant marker of DNA oxidation. In 1998, Kamiya et al. demonstrated that the mutagenicity induced by 2-OH-dATP in E. coli was more significant than that caused by 8-OH-dGTP, which also gave a confirmation of 2-OH-dATP-G base pair.

Interestingly, the nonstandard isoG–isoC base pair contributes to the stability of duplexes. In 2001, Turner et al. reported this effect has a sequence dependency in duplexes because of the large difference in electron density. In 2004, Prudent et al. proved that isoG–isoC stabilizes the G–C double helix and can be applied to polymerase chain reaction (PCR) to detect the
mutant genes. Beyond that, in 1992, Benner et al. raised that it can help regulate ribosome translation. 91% of isoG-containing RNA could be read, which is higher than the control, because the frameshift with isoG skips the isoC. However, the transfer RNA (tRNA) containing the meaningless CUA codon is meaningless and results in translation termination. This property can also aid the development of nonstandard base pairs to extend gene dictionaries and provide a deeper understanding of translation termination. Moreover, in 1993, Switzer et al. reported that the tautomerism of isoG leads to various mispairings, which may enhance the antiviral activity. The stability of base-pairs of z8isoGd (46, Fig. 10) with 5-methylisoC, Ad, Td, Cd and Gd can be monitored by fluorescence, in other words, the fluorescence signal is correlated with DNA base pairing stability. Thus, z8isoGd can be applied as a sensor with fluorescence for detecting mismatch DNA duplexes, even more complicated DNA structures, such as in triplexes reported by Seela in 2010.

6.3 Applications of 1-methyl-isoG

In 1981, Fuhrman et al. reported that isoG presents negative inotropic and chronotropic effects in the myocardium. And those effects can be antagonized by theophylline. The effects of negative inotropic and chronotropic activity ranked in the order isoG > 1-methyl-isoG > adenosine > 2-methoxyadenosine. And these substances may act on the same receptor. In addition, as reported by Emerson et al. in 1949 and Brown et al. in 1952, isoG can produce smooth muscle relaxation, hypotension and anti-inflammatory, activating adenosine cyclase (AC) and anti-allergic effects (Fig. 22). In 1981, as shown in Table 5, a comparison of effects of the derivatives of 1-methyl-isoG have been explored by Cook et al. Moreover, the effects of halogenated analog 2-chloroadenosine are greater.

These effects induced by 1-methyl-isoG are more potent than adenosine because 1-methyl-isoG can resist the action of adenosine deaminase. So 1-methyl-isoG can exist in the extracellular fluid for a long time, whereas adenosine has been degraded into inactive inosine. And there is a positive correlation between the potency of resistance to adenosine deaminase and the muscle relaxation and cardiovascular effects. However, in 1985, Radulovacki et al. reported 1-methyl-isoG does not have a strong hypnosis effect similar to...
adenosine. And, in 1981, Spence et al. showed that the effects of 1-methyl-isoG and adenosine on neuromuscular transmission in the vas deferens are similar. This is probably because the enzyme activity of adenosine deaminase in the vas deferens is inherently low, and thus the degradation is low. The ability of the enzyme activity of adenosine deaminase in the vas deferens are similar. This is probably because 1-methyl-isoG and adenosine on neuromuscular transmission in the vas deferens are similar. This is probably because the enzyme activity of adenosine deaminase in the vas deferens is inherently low, and thus the degradation is low. The ability of 1-methyl-isoG to cause the accumulation of cyclic adenosine monophosphate (cAMP) in brain is not e

isoG to cause the accumulation of cyclic adenosine monophosphate (cAMP) in brain is not effective as that of adenosine, but neither of these two nucleosides show inhibition of rat brain cAMP phosphodiesterase (PDE). Besides, in 1980, Taylor et al. proved that accumulated adenylate cyclase (AC) caused by 1-methyl-isoG may be associated with cardiovascular/relaxation effects.

In 1981, Marwood et al. showed that 1-methyl-isoG has equal negative inotropic and chronotropic effects in guinea-pig isolated atria. Nevertheless, adenosine has a greater negative inotropic effect. The active site of 1-methyl-isoG is only on the atria, whereas adenosine can also act on ventricle. Furthermore, 1-methyl-isoG showed a biphasic response on cardiac output, which means a slow increase followed by a rapid initial decrease. The impact of 1-methyl-isoG in lowering response is not experimented.

### Table 5: Comparisons of the effectiveness of 1-methyl-isoG and its derivatives in muscle relaxation, hypothermia, hypertension, anti-inflammatory and anti-allergy effects

| Number | Muscle relaxation | Hypothermia | Hypotension | Anti-inflammatory | Anti-allergic |
|--------|------------------|-------------|-------------|------------------|--------------|
| 37     | Y*              | Y*          | Y*          | Y*               | Y*           |
| 38a    | Y*              | Y*          | Y*          | Weakb           | Y*           |
| 39g    | N'              | N'          | Y*          | Weakb           | Y*           |
| 39h    | N'              | N'          | Weakb       | _d              | Y*           |
| 39i    | N'              | N'          | _d          | _d              | Y*           |
| 39j    | N'              | N'          | _d          | _d              | Weakeb       |
| 40a    | N'              | N'          | _d          | _d              | Y*           |
| 40b    | N'              | N'          | Weakb       | _d              | _d           |
| 40c    | N'              | N'          | Weakb       | _d              | _d           |
| 54a    | N'              | N'          | _d          | _d              | _d           |
| 54b    | N'              | N'          | _d          | _d              | _d           |
| 54c    | Y*              | Y*          | Y*          | Y*               | Y*           |
| 55     | N'              | N'          | _d          | Weakb           | Y*           |
| 56     | Y*              | Y*          | _d          | _d              | Weakeb       |

*a Effective, b Limited effect, c Invalid, d Not experimented.

In 1980, Taylor et al. reported 1-methyl-isoG does not compete with adenosine for uptake sites to activate adenosine cyclase. This indicates that it does not attenuate the effect of adenosine by competition, but because 1-methyl-isoG is not degraded and remains for a long time in the extracellular fluid. 1-Methyl-isoG was thought to act on a benzodiazepine receptor, but that the hypnotic effect was not the same as benzodiazepine ruled that out by Hall et al. in 1987. Those effects above are dose-dependent. Moreover, theophylline, a widely-used blocker of adenosine receptors, also acts as a dose-dependent antagonist of the effects of 1-methyl-isoG. So 1-methyl-isoG may act on the same receptor as adenosine in the intestinal smooth muscle and pig brain.

### 6.4 Ionophores

Nuclear waste remnants from the Second World War, nuclear testing, and the nuclear industry have left a large amount of radioactive contamination, mostly Cs$^+$ and Ra$^{2+}$. They are challenging to remove, leaving behind huge potential risk for human being and pollution of water and soil. Compounds that are highly selective for Cs$^+$ are required because of the large quantity of Na$^+$ and K$^+$ in nuclear waste. In 1990s, crown ethers have been developed to bind Cs$^+$ by McDowell et al. and Schuleben et al. But the Cs$^+$ selectivity are modest resulting from their flexibility. So rigid macrocycles, especially calixarene crowns and their derivatives with different binding groups attached, have been used as recycling ligands. Rigid macrocycles show better complexation and selectivity for Cs$^+$ over other ions such as Li$^+$, Na$^+$, K$^+$ and Rb$^{+}$. In addition, in 1995, calixarene carriers were reported to have rattleing cell membrane penetration in polymer inclusion membrane (PIMs) by Reinhoutr et al. However, these macrocycles are expensive to synthesize and the ionophore recovery, as well as cations release, are difficult. Nowadays, noncovalent ionophores containing isoG are widely used. They show greater affinity and selectivity for Cs$^+$ compared to those of calixarene and its derivatives. In 1997, Davis et al. reported the self-assembled (isoG)$_{10}$ M$^+$ ionophore shows significant selectivity for Cs$^+$ in

- [Image 14x290 to 26x354]

**Table 5:** Comparisons of the effectiveness of 1-methyl-isoG and its derivatives in muscle relaxation, hypothermia, hypertension, anti-inflammatory and anti-allergy effects.
competition experiments. And (isoG)10-M+ can even extract bound Cs+ from a calixarene crowns ether. Thus, these compounds can be used for purification and metal nuclear waste disposal.

Further, in 2000, Davis et al. then reported that in the (isoG)10-Cs+ system (Fig. 15 and 16), Cs+ and the protected isoG 53 are in constant exchange with the outside world. However, the exchange rate of Cs+ itself is 40 000 times faster than the compound 53, as shown by multinuclear NMR spectroscopy. So the ion carrier does not disintegrate during ion exchange. The ions can be easily dissociated from the noncovalent ionophore when the solvent polarity changes. The same exchange process is true for other metal ions. Specially, Cs+ possesses an advantage that it can even slow down the isoG exchange rate. In 2001, they proved that the protected isoG 53 assembly can not only release Cs+ more easily without dissociation, but also show a stronger affinity for larger cations such as Cs+ and Ba2+ than K+, Sr2+ and Na+. They also demonstrated that [53]p-Cs+ can also promote selective transport through organic polymer inclusion membrane (PIMs) and bulk liquid membranes (BIMs), with high transport rates and excellent selectivity for Cs+. This cation-binding selectivity is attributed to the geometric hydrogen-bonding mode composed of donors and receptors. As discussed earlier, G forms G-quartets, whereas isoG prefers the formation of pentads. The sizes of these macrocyclic cavities of self-assembled ionophores determine the cation-binding selectivity. Moreover, in 2004, they reported that in the case of G, the selectivity for Ra2+ prior to magnesium ion (Mg2+) and calcium ion (Ca2+) requires an existing lipophilic anion.192 Nevertheless, isoG shows preferential selectivity for Cs+ over Mg2+, Ca2+, Sr2+ and Ba2+ without any additional environmental conditions. In addition, a significant radium ion (226Ra2+) preference over other monovalent alkali cations was observed for both 57 and 53, with a “precipitation prevention/dissolving” property over a wide pH range. And, in 2005, Reinholdt et al. reported that Ra2+ can be selectively extracted from gas-field produced water by isoG-based self-assemblies.

6.6 More applications

Apart from applications in gel formation, ionophore, and as antitumor agent, in 1973, isoG was reported to be an inhibitor of inosine monophosphate (IMP) pyrophosphorylase by Hagen.196 And in 1974, Holy et al. reported that isoG can also inhibit inducible E. coli binding site. In addition, in 1975, Mantsch et al. reported that its 5′-di- and -tri-phosphates can strongly bind and inhibit glutamic acid dehydrogenase. In 1988, Wissler et al. reported that isoG also acts as an essential constituent of Cu(II)-containing ribonucleic acid produced by cultured pig macrophages. This unusual Cu(n)-containing extracellular RNA was a formidable angiogenesis factor, and acted as an adenosine A1-receptor agonist. Besides, in 1999, Davis et al. raised that the protected isoG (53, Fig. 11) self-assembly can act as phase transfer catalysts. And in 2018, Anna et al. proposed that by strongly affecting the highly ordered structure of the G-quartet in TBA, isoG greatly affects the anticoagulant activity of the thrombin binding aptamer (TBA).

7 Summary and outlook

In summary, since Fischer first synthesized isoguanine in 1897, there have been many reports focusing on the comparison of isoG with G. In this review, we have detailed the discovery, and synthesis of base- or sugar-modified derivatives of isoG, its monomer structure and aggregates, and the diverse applications of isoG/isoguanine. Since the yields of isoG/isoguanine in natural sources such as croton beans, butterfly wings and marine mollusk are too low, a series of synthetic methods of isoG/isoguanine by introducing functional groups to the heterocycle of G and AICA have been proposed. And various base- or sugar-modified derivatives have been synthesized or found in nature. Additionally, some unique properties of isoG and its derivatives including enol–keto tautomerism, mispairing, self assembly into tetramer (isoG4), decamer (isoG10) and isoG quadruplex, and fluorescence suggest its potential diverse applications in the formation of supramolecular hydrogels, in ps and aps duplexes and even triplexes, also in waste-collection as an ionophore. In addition, further applications such as antitumor activity, inhibition of IMP-pyrophosphorylase, and mutation-inducing have been talked about. As the structure and properties of isoG become better understood, there will be further studies and more applications will be identified. For example, because of its unique hydrogen bond acceptors and donors, isoG is expected to have a wider range of applications in the field of supramolecular self-assembly, the formation of hydrogel and ionophore. Additionally, iso6 is most likely to play a significant role in genetic disease monitoring probes because of the variable nonstandard pairs caused by its tautomerism and its incorporation by different enzymes. Moreover, benefited from its antitumor ability and self-assembly properties, the
preparation of supramolecular gels integrating antitumor activity and drug delivery appears extremely significant. Thus, isoG may play a significant role in the clinical treatment of various cancers and other therapies involving smooth muscle relaxation, hypotension, and bradycardia. In conclusion, the unique properties of isoG, such as its use in supramolecular assemblies, as a nonstandard base and a medicine with antitumor activity, will broaden its applications in diverse fields.

**Conflicts of interest**

There are no conflicts to declare.

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